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The synthesis of endothiopeptides.

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THE SYNTHESIS OF ENDOTHIOPEPTIDES

submitted by CLIVE VICTOR WALKER

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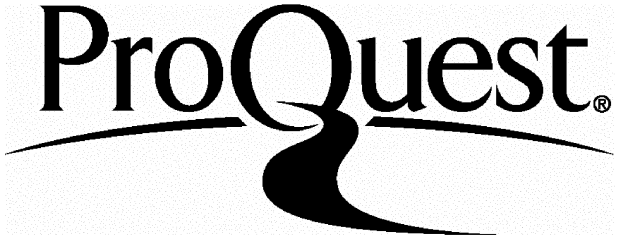
Clive Walker



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ABSTRACT

Synthetic approaches to Endothiopeptides where the thioamide group has replaced the normal amide link are discussed and investigated.

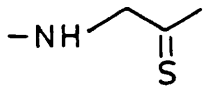
A range of N-protected dipeptide esters (21) has been thionated exclusively, and in high yield, at the amide carbonyl using Lawesson's reagent (25) giving synthetically useful protected endothiodipeptides (55). Investigation of more conventional peptide methodology and its application to these new systems has been carried out.

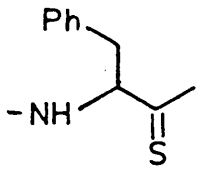
Deprotection of the N-terminus of the endothiodipeptides (55) gives, after coupling, larger tripeptides with a single thioamide link. Extension of the C-terminus is achieved using the phenyl ester as an active ester group, thus avoiding a deprotection step. In this fashion the protected pentapeptide Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87) has been prepared by two routes and represents a monothionated leucine enkephalin analogue. The fully deprotected pentapeptide (83) has been obtained via an alternative derivative, BOC-Tyr(BOC)-Glyt-Gly-Phe-Leu-OBu^t (94) in a single step, and may have greater and longer lasting analgesic activity than its normal oxygen counterpart.

Approaches to the synthesis of novel endothioaziridine peptides are explored. Milder thionation methods are needed to obtain these systems owing to the nature of the aziridine ring. Several direct thionation methods and indirect methods for incorporation of the thionation functionality are investigated.

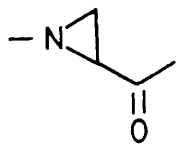
NOMENCLATURE AND ABBREVIATIONS

Chiral amino acids are of S stereochemistry unless otherwise stated. Amino acid and protecting group abbreviations are those recommended by IUPAC.[122] The thiocarbonyl derivative is designated by the 't' symbol after the appropriate amino acid abbreviation.[33a] Thus,

Glyt = 

Phet = 

Other abbreviations used in the text are as follows:

ACE	Angiotensin Converting Enzyme
Azy	Aziridinyl 
CI	Chemical ionization
CPA	Carboxypeptidase A
DCC	N,N'-dicyclohexylcarbodiimide
DCM	Dichloromethane
DCU	N,N'-dicyclohexylurea
DMSO	Dimethylsulphoxide
DPPCl	Diphenylphosphinyl chloride

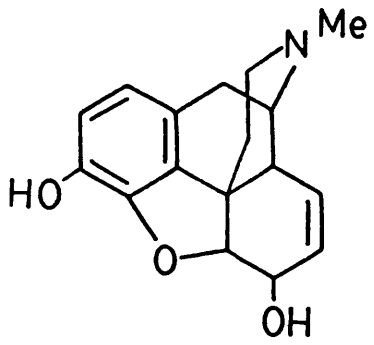
FAB	Fast Atom Bombardment
IPA	*Iso*-propyl alcohol
IR	Infra Red
m/e	Mass to charge ratio
mp	Melting point
MS	Mass spectrum
NMM	N-methyl morpholine
n.m.r.	Nuclear magnetic resonance
n Oe	Nuclear Overhauser effect
TEA	Triethylamine
TFA	Trifluoroacetic acid
TFMSA	Trifluoromethanesulphonic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultraviolet

CONTENTS

INTRODUCTION

1.1. BACKGROUND

The understanding of the mechanisms of pain and thus its treatment has been one of the earliest medicinal and pharmacological objectives. A large number of pain relieving (analgesic) drugs are known of which morphine (1) is probably still the most important and the most clinically effective.

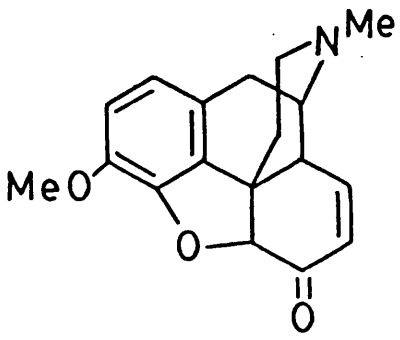


(1)

Morphine acts on the central nervous system (CNS) unlike the "peripheral" antiinflammatory analgesics such as acetylsalicylic acid (Aspirin). The medicinal chemistry, pharmacology and toxicology of morphine and related compounds have been extensively studied.[1]

An essential feature of medicinal chemistry is the development of drugs with improved efficacy and less undesirable side effects.[2] A wide range of morphine analogues have been synthesized, tested and, in some cases, marketed, for example dihydrocodeinone (Trade Names, Codone, Dicodid) (2) and Naloxone,[3] (3).

Despite the extensive research in this area[3] it is doubtful whether any other drug is clinically *more* effective than morphine, the



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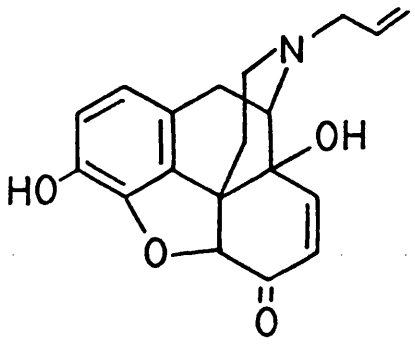


(3)

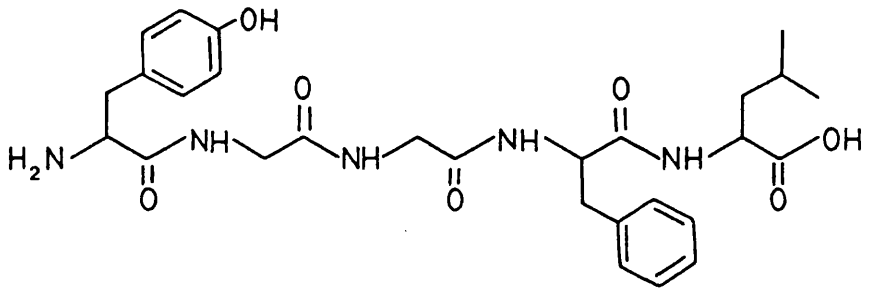
standard against which other compounds are measured. Due to its serious side effects, however, it is restricted mainly to patients with severe pain.[1]

In Man morphine acts in a narcotic fashion, inducing analgesia, drowsiness, changes in mood (euphoria and dysphoria) and mental clouding. Minor side effects are constipation and pupil constriction but more serious drawbacks are respiratory depression, vomiting, nausea and, after prolonged use, drug tolerance and addiction.[1] Although these side effects may be as a result of the opiate mode of action (through the CNS) there is clearly a need for a non-addictive, centrally acting analgesic with little or no side effects (!) but as yet none has emerged.

Table 1 Amino Acid sequences of the Endorphins

Peptide	Sequence	β-lipotropin residues
Met-enkephalin (4)	Tyr-Gly-Gly-Phe-Met	61-65
Leu-enkephalin (5)	Tyr-Gly-Gly-Phe-Leu	-
α-Neoendorphin	(5)-Arg-Lys-Arg	-
α-Endorphin	(4)-Thr-Ser-Glu-Lys-Ser Gln-Thr-Pro-Leu-Val-Thr	61-76
γ-Endorphin	61-76-Leu	61-77
β-Endorphin	61-77-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu	61-91

(4)



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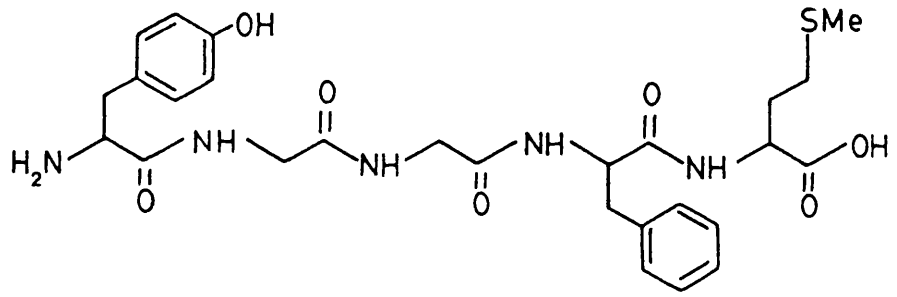


1.2. OPIOID PEPTIDES

The Enkephalins (4 and 5) are endogenous pentapeptides with opioid properties. They belong to a class of neuropeptides found in the brain called the Endorphins which are (mostly) structurally related to a specific peptide sequence of the pituitary hormone β-lipotropin (Table 1).[4]

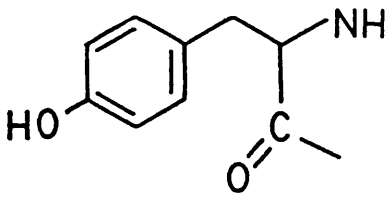
The opioid peptides show similar pharmacological effects to the morphine alkaloids. They act as inhibitory neurotransmitters affecting acetylcholine and norepinephrine release.[4] Their analgesic effect, however, is short lived presumably due to inactivation within the brain by 'peptidase' enzymes (see later). The control of many other nervous system functions are mediated by the endorphins. They can act as inhibitors and modifiers of pleasure, sleep, sexuality, eating and drinking, and also in the autonomic control of other body functions (i.e. blood pressure and respiration).[4,5]

The enkephalins were the first opioid peptides to be isolated and structurally elucidated. This was achieved by Hughes *et al.*[6] (1975) after a search by other groups.[7] The rationale behind this research stems from the conclusion that morphine itself must interact with specific brain receptors.[1,2,8,9] For instance, of all the morphine-like compounds, with similar clinical effects, there are several common structural features present. These include an aromatic ring and a basic nitrogen atom at a distance from it of 2-3 carbon atoms. Small structural alterations lead to a dramatic decrease in activity. Morphine analogues can also antagonise its action,[10] for example Naloxone (3).[11] Such antagonists probably interact with the same receptors as morphine in order to cancel or partially block its action. Receptors of this type must have been designed to interact with

compounds other than morphine since most animals do not come into contact with opium poppies (!)

The enkephalins (and endorphins) have been shown to interact with similar receptors[12] and there are clear structural similarities between the tyrosyl amino acid (6) in the enkephalins and the morphinoid phenol ring (Figure 1).

Figure 1



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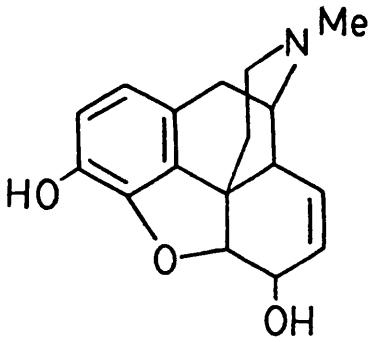


(1)

In addition it is probable that the enkephalins can assume a conformation at the receptor(s) that closely mimics the rigid morphine-like structure.[13]

The important twin bioassays used to assess morphine for opiate activity in novel, potential analgesics are the Mouse Vas Deferens (MVD) and Guinea Pig Ileum (GPI) tissue preparations.[14] The tissue is

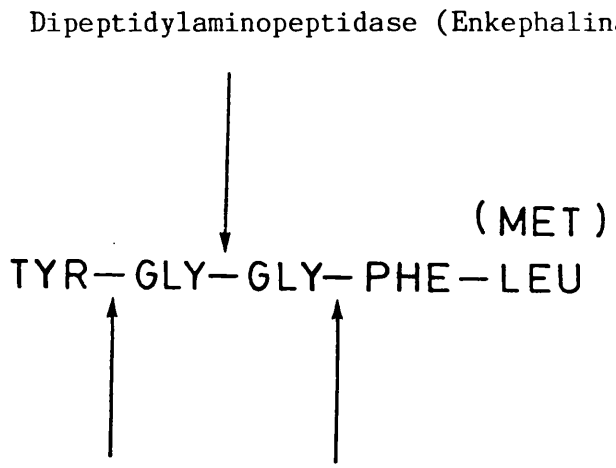
electrically stimulated and the depression of the contractions by opiates is measured. The two preparations have been shown to contain a mixture of receptors[12a,15] and the enkephalin activity in these two systems, controlled by specific receptor interactions,[12a] is different due to the non-identical nature of the receptor populations. In the MVD case the enkephalins interact with "δ-receptors" predominantly but also affect the "μ-receptors" to a lesser degree. Conversely in the GPI system the opioid peptides interact with "μ-receptors" and their activity is much less. Thus, it seems that "morphine receptors" are of the so-called μ-type whereas "enkephalin receptors" are the δ-type. From this one would expect morphine and analogues to be more active in the GPI assay while the enkephalins should be more potent in the MVD test and this is indeed the case.[15] Other receptors (σ, K and ε) have also been proposed as being implicated in opiate and enkephalin binding activity.[16,8b]

1.3. ENKEPHALIN ACTIVITY AND "ENKEPHALINASES"

Although many enkephalin analogues have been synthesized and assessed *in vitro*[5,17,18] and their interactions with specific receptors studied,[19] their *in vivo* biological activity is short-lived. This may be attributed to poor passage through the blood-brain barrier[20] and their susceptibility towards enzymic degradation after administration.[21,22] All but one of the amide bonds in leucine or methionine enkephalin may be hydrolysed enzymatically[21] (Figure 2).

The most important sites of enzymic action are the Tyr-Gly amide link (although it has been suggested that this is not important in the inactivation of enkephalins at the synaptic clefts[21]) and the Gly-Phe bond. Cleavage of the Gly-Gly bond by "Enkephalinase B" has so far

Figure 2



not been as well characterized as the other sites. Clearly, the development of more *in vivo* active enkephalin analogues requires improvement of their enkephalinase resistance without altering the specific receptor activity.[18a,23] Alternatively the synthesis of inhibitors, or substances that will bind to the enzyme's active site irreversibly ("suicide substrates") has also been carried out.[22]

Based on analogy with the action of Carboxypeptidase A[24] (CPA) an active site model for Enkephalinase A is shown in Figure 3 below.[21]

(7)

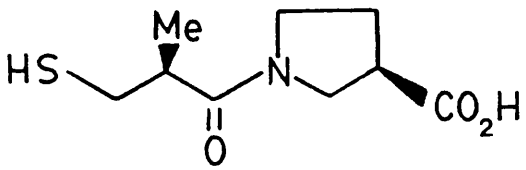
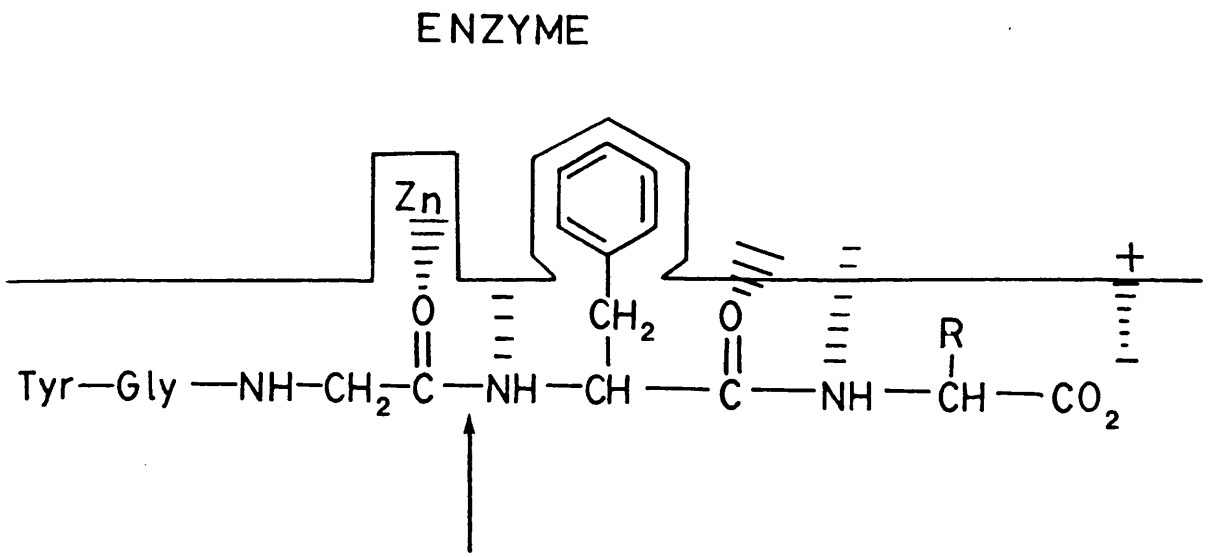


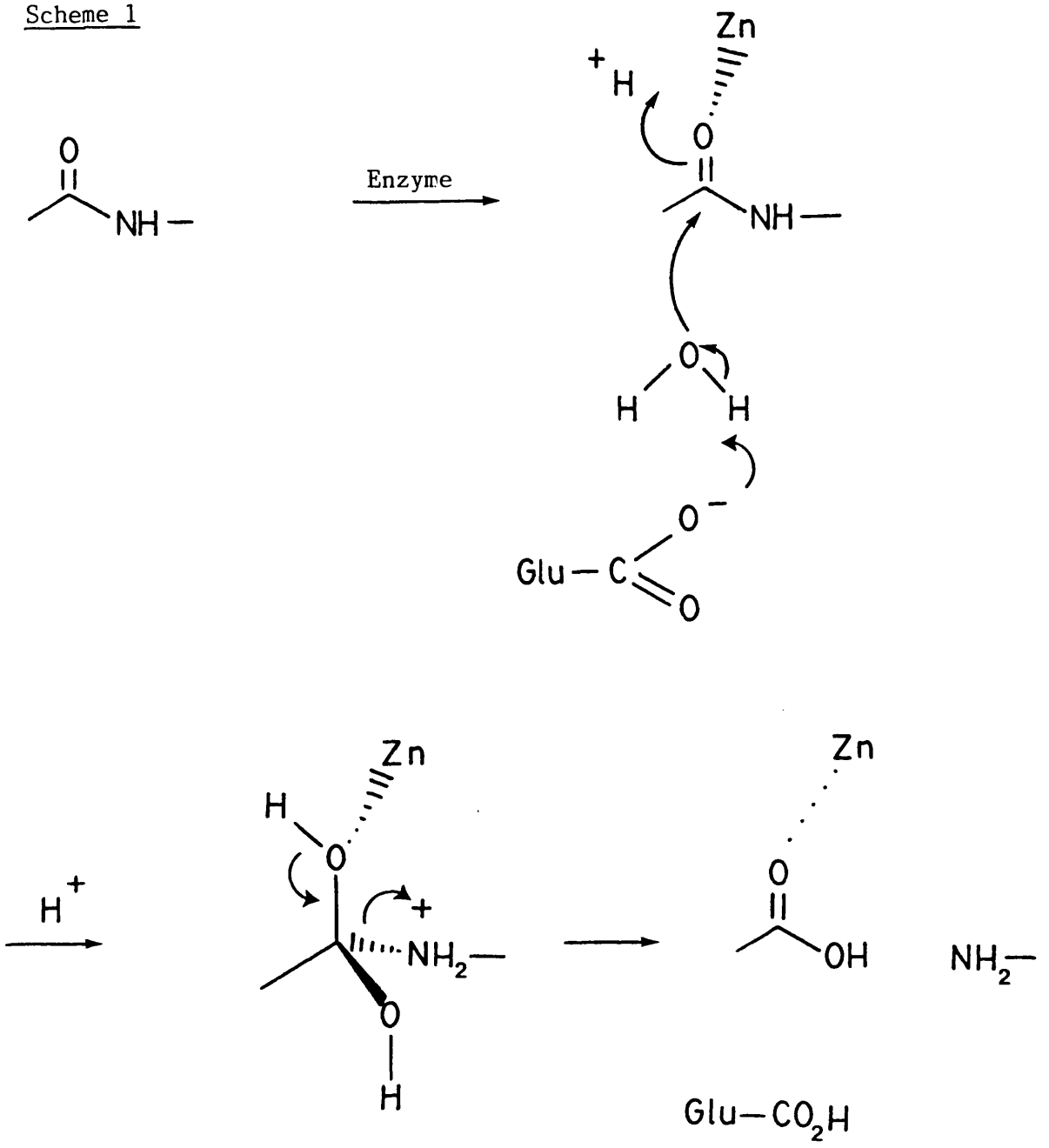
Figure 3



The development of successful inhibitors of Angiotensin converting enzyme (ACE) based on a similar model has led to the anti-hypertensive agent Captopril (7)[24b,25] and other inhibitors based on by-product analogy have also been studied (for example, L-benzyl-succinic acid[26]). A related model may exist for the Gly-Gly bond cleavage case ("Enkephalinase" B).

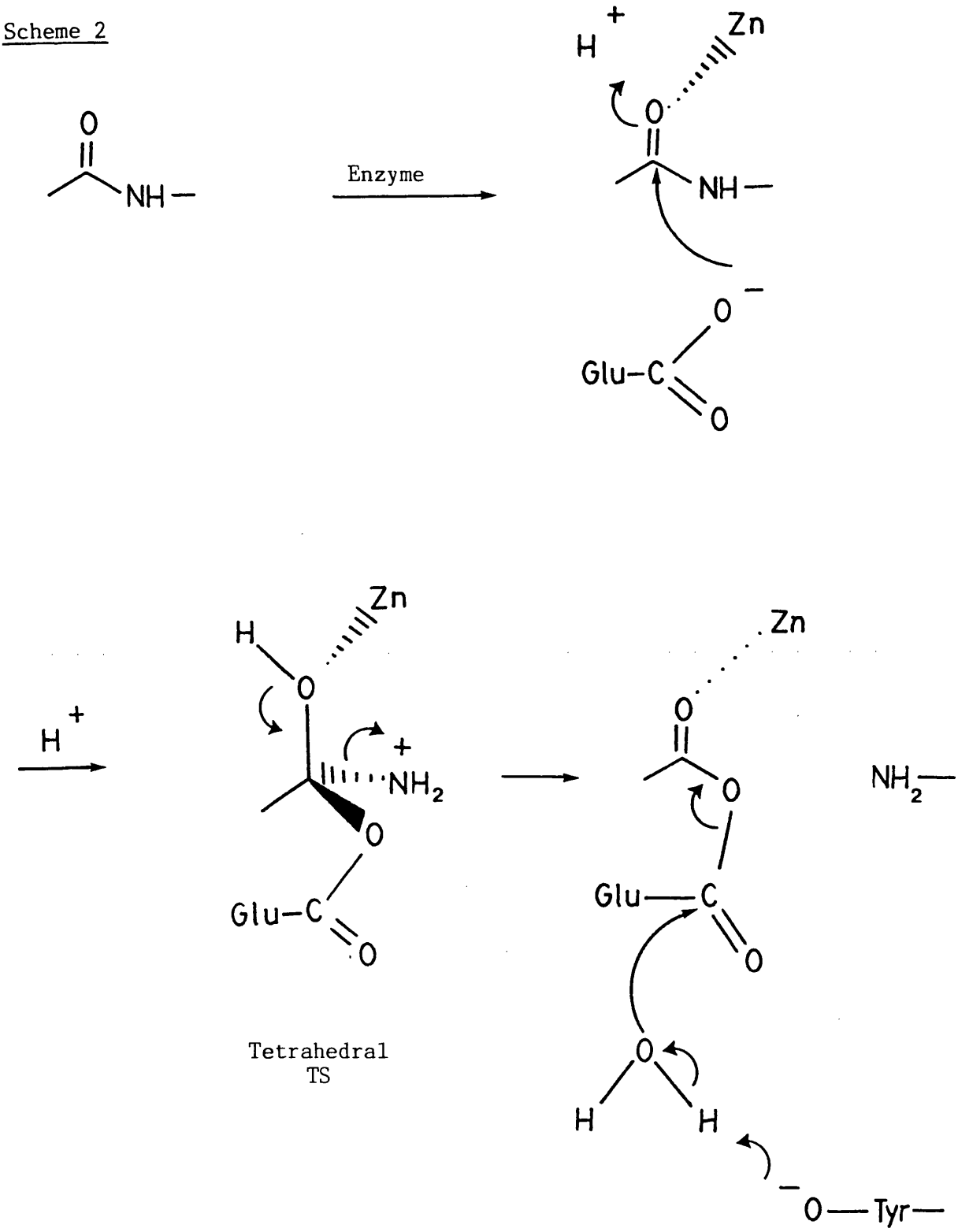
Carboxypeptidase A which is a related enzyme to the dipeptidyl Enkephalinases A and B, in that it is a zinc containing enzyme operating on peptide amide bonds, has been proposed as having two major probable mechanisms of action.[24a] These are shown below in Schemes 1 and 2.

Scheme 1



For both schemes binding to the zinc atom of the enzyme serves to polarize the scissile amide bond and may also twist it out of plane.[27] Both these effects make it more susceptible to attack by nucleophiles.[24a,28] In Scheme 1 the glutamic carboxylate group (present on the enzyme active site) promotes the attack of a water molecule on the amide bond giving a tetrahedral transition state. Abstraction of a proton (from a tyrosyl amino acid on the enzymatic active site) leads

Scheme 2



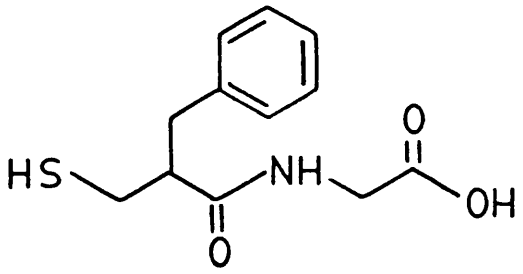
to amide bond cleavage.

For the second scheme, the glutamic carboxylate moiety again plays a prominent role. Attack on the polarized amide bond gives an anhydride type tetrahedral transition state. Following a similar path to Scheme 1, this cleaves to an acyl-enzyme intermediate (which can be later attacked by a water molecule giving two separate carboxyl groups) and the free amino peptide.

Both mechanisms have points in their favour but further studies are necessary to resolve the ambiguity. It may be that different substrates are cleaved according to different mechanisms.[29] The main features of both are shown in Scheme 3.

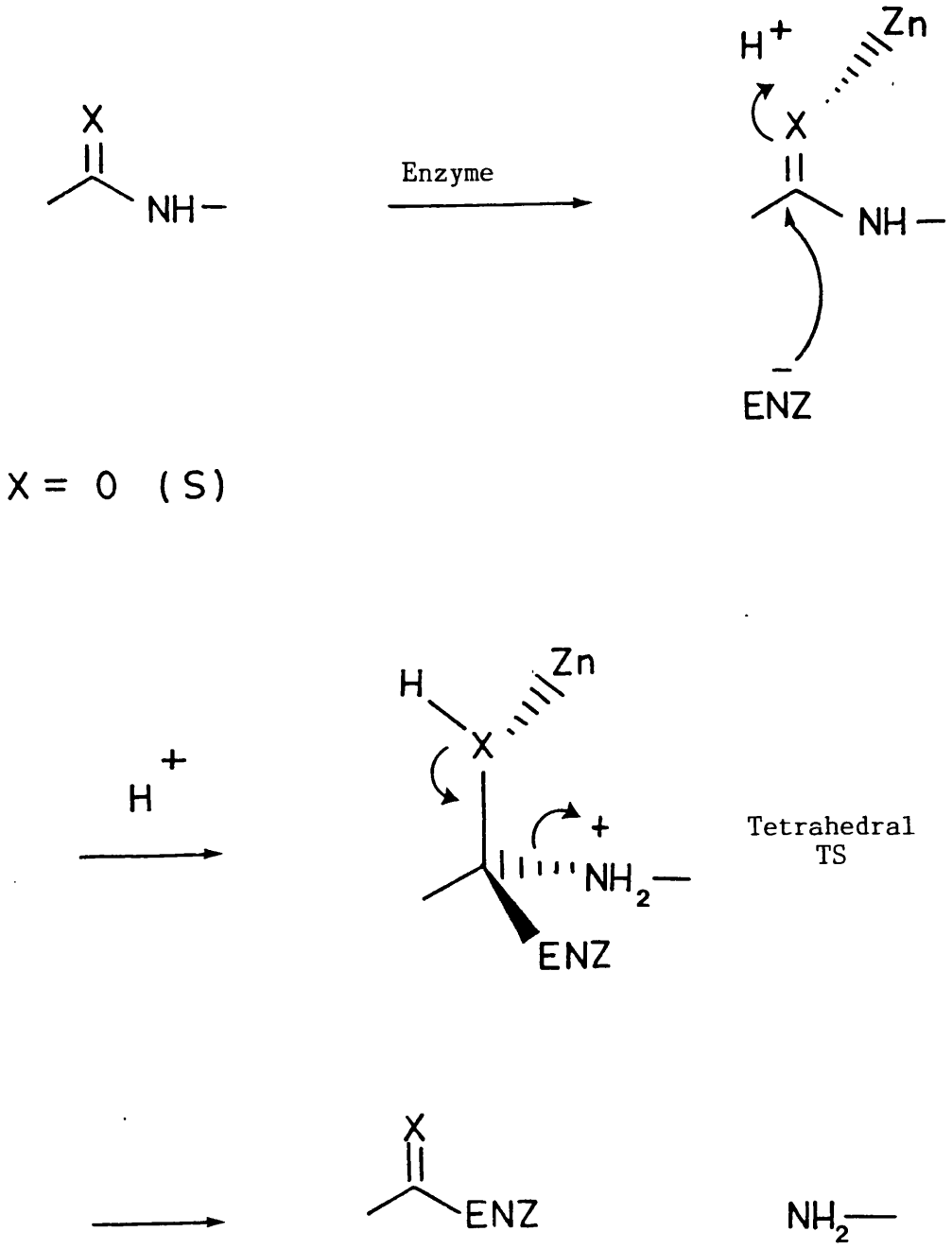
Any functionality at the scissile position that can coordinate more strongly than oxygen may irreversibly bind to the enzyme. This is probably the case for Captopril, (7), and ACE. The potent enkephalinase inhibitor Thiorphan, (8)[5] ((DL)-3-mercapto-2-benzylpropanoylglycine) can also bind to zinc in this fashion.

(8)



Other functional groups required for activity in the enkephalins also fit the active site model.[18b] For example the phenyl ring of the

Scheme 3



phenylalanyl amino acid is accommodated in a hydrophobic "pocket" of the enzyme. The anionic carboxylate group interacts with an oppositely charged amino acid (possibly arginine).

These features would need to be present in any potential inhibitor for it to be "recognised" by the enzyme. Thus a molecule containing the minimum structural features necessary to obtain enzymatic resistance without losing pharmacological activity and/or

receptor specificity[18a] would be the desired compromise. Other enkephalin analogues with these characteristics have been investigated. For example, the incorporation of specific dehydroamino acids into the enkephalin sequence at certain positions has produced analogues that are resistant to enzymatic hydrolysis.[17,18a] Although these analogues are hydrolysed slowly by carboxypeptidase enzymes *in vitro* and have high δ-receptor specificity, enabling the enkephalin receptor-substrate interactions to be more fully explored, their analgesic effect is weak. Possibly, this is due to their high δ-receptor activity, i.e. the analgesic effect may be more strongly mediated through the μ-receptors.[18a] The concept of one receptor producing a specific response is difficult to substantiate although one receptor may be more responsible for one effect rather than another.[30]

1.4. ENDOTHIOPEPTIDES

1.4.A Amides and Thioamides

The selective replacement of amide bonds by the thioamide[31] moiety which is expected to have similar steric and electronic requirements to its oxygen counterpart may have the desired effect of combining enzymatic resistance with analgesic activity. Coordination of the thioamide sulphur atom to the metal (zinc) could lead to more efficient substrate-enzyme binding (the affinity of sulphur for divalent metal cations is well known[32]) yet the common structural requirements could equally yield analgesic potency. Furthermore, attack at the thiocarbonyl group will produce a tetrahedral intermediate, orders of magnitude more stable than that for the natural substrate, and a relatively stable tetrahedral transition state surrogate could be formed.

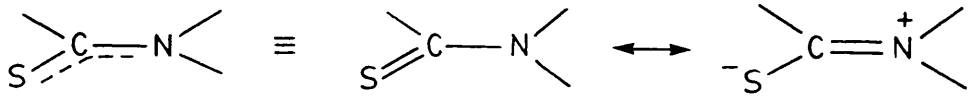
X-Ray studies[31a] have shown that the atoms of the thioamide bond are in a plane and spaced rather like an olefinic bond with bond angles of ~120°. The larger covalent radius of sulphur ($1{\cdot}04 \times 10^{-10}$m) compared to oxygen ($0{\cdot}74 \times 10^{-10}$m) means that the carbon-sulphur bond in thioamides ($1{\cdot}7 \times 10^{-10}$m) is longer than in ordinary amides ($1{\cdot}25 \times 10^{-10}$m) but the length of the carbon-nitrogen bond is about the same.[31a]

Both the carbon-sulphur and carbon-nitrogen bonds are intermediate in length between single and double bonds (Table 2). This

Table 2 Comparison of bond lengths

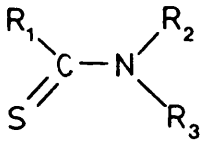
Bond	Length ($\times 10^{-10}$m)		
	Single	Double	Thioamide (amide)
C-S	1·81	1·60	1·7 (1·24)
C-N	1·47	1·27	1·35 (1·34)

suggests appreciable electron delocalization (9a) and much sp^2 character in the carbon-nitrogen bond (in the valence bond theory this is analogous with the charged species (9c)).



(9a) (9b) (9c)

The energy of activation needed for rotation about the carbon-nitrogen bond is greater in thioamides than that in amides.[31a] The non-equivalence of alkyl groups attached to nitrogen and the existence of conformational bias in thioamides has been established by n.m.r. techniques. Generally the Z-isomer predominates and for



$S > R_1$
$R_2 > R_3$

E-isomer

secondary thioformamides dipole moment considerations have been used to explain the Z-isomer preference.[31a] The larger steric bulk of the sulphur atom means that the Z-isomer is generally less favoured for thioamides than for amides.

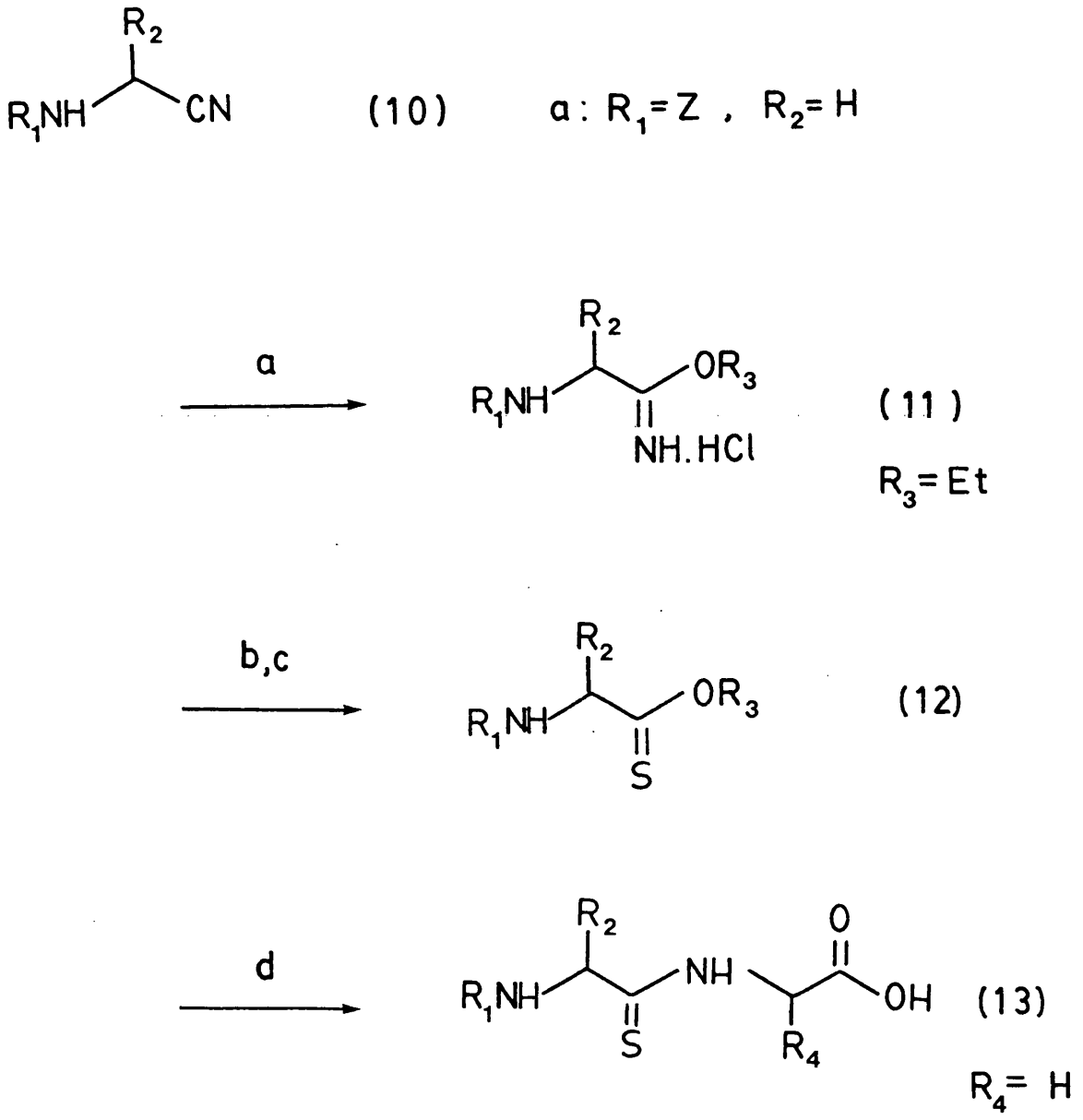
Two mesomeric forms for thioamides have been suggested (9b and 9c). The thioimidate form (9c) is proposed on grounds of chemical reactivity and increased acidity of thioamides compared to amides. However, there is no or little spectroscopic evidence for its existence. X-Ray diffraction and fluorescence, n.m.r., IR and UV techniques have shown no evidence for the imidothiol form[31a] and clearly there is insufficient of it present to allow detection by these techniques.

1.4.B. Literature review

Surprisingly, until recently the chemistry and synthesis of endothiopeptides[33] had not been extensively explored. The earliest

report of their synthesis is by Ried and von der Emden,[34] and by the same authors in 1961,[35] and these reports had not been followed up. Their route to endothiodipeptide acids is shown in Scheme 4.

Scheme 4



a, R_3OH, HCl; b, K_2CO_3; c, H_2S, 18 h, RT; d, NaOH, glycine.

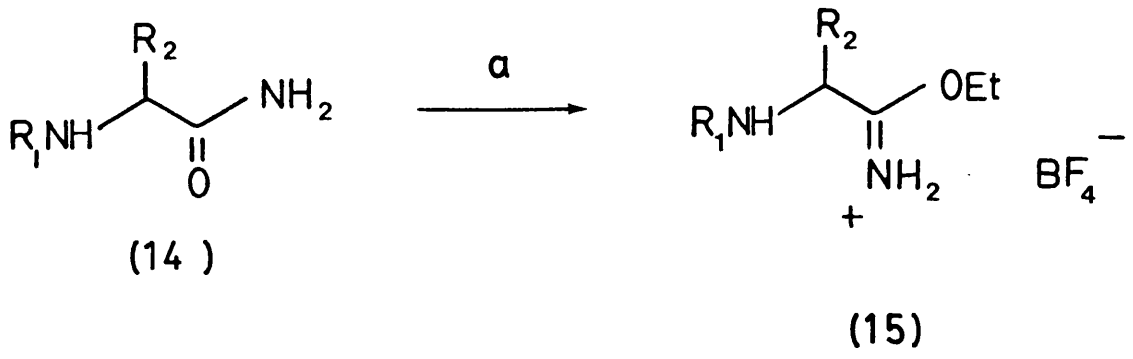
N-Protected α-aminonitriles, (10), obtained via the Strecker synthesis[36] from the aldehyde, hydrogen cyanide and ammonia, then N-protection, were converted to the amino acid imidoester hydrochlorides (11)[37] with ethanolic hydrochloric acid. Thionation with hydrogen sulphide, after desalting with potassium carbonate, at room temperature (18 h) gave the key intermediate N-protected α-amino acid thionoesters (12). The latter were successfully coupled to other amino acids by sodium hydroxide treatment. Four examples of N-protected endothiodipeptide acids, (13), were prepared in this way.

Subsequently Ried and Schmidt[38] (1966) tried a different route to obtain the thionoesters (12) whereby the N-protected amino acid amide (14) (obtained from the acid chloride via ammonia treatment) is refluxed with triethyloxonium tetrafluoroborate giving the salts (15) (Scheme 5).

This route used enantiomerically pure amino acid amides in some examples but no assessment of the optical integrity of the product(s) is made. An improved route to (12) was tried and the final step utilized triethylamine (TEA) rather than sodium hydroxide as a base. Although yields were moderate-to-good both routes used inconvenient and hazardous reagents (hydrogen sulphide), several steps and prolonged reaction times and thus accentuated racemization problems.

More recently Mock *et al.*[39] (1981) and Campbell and Nashed[40] (1982) prepared the tripeptide (19) (Scheme 6) but used the dithioester (17) instead of the thionoesters (12) used by Ried and co-workers.[34,35,38] Acid treatment of (19) gave the free acid (20).

Scheme 5

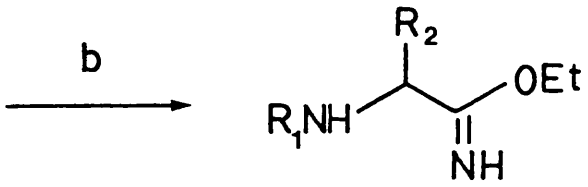


(14)

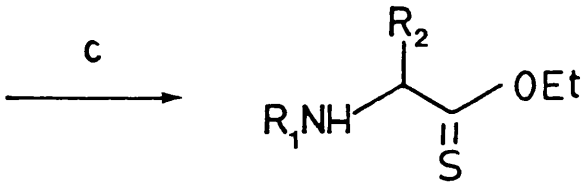
(15)

R_1 = Z, Ts, Pht

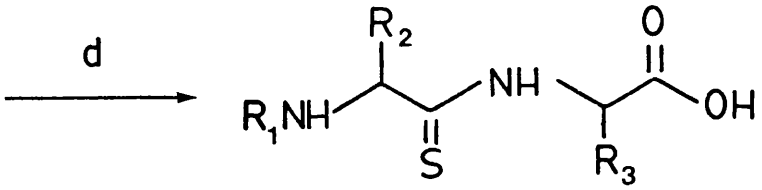
R_2 = Me, CHMe, s-Bu, Bzl



(16)



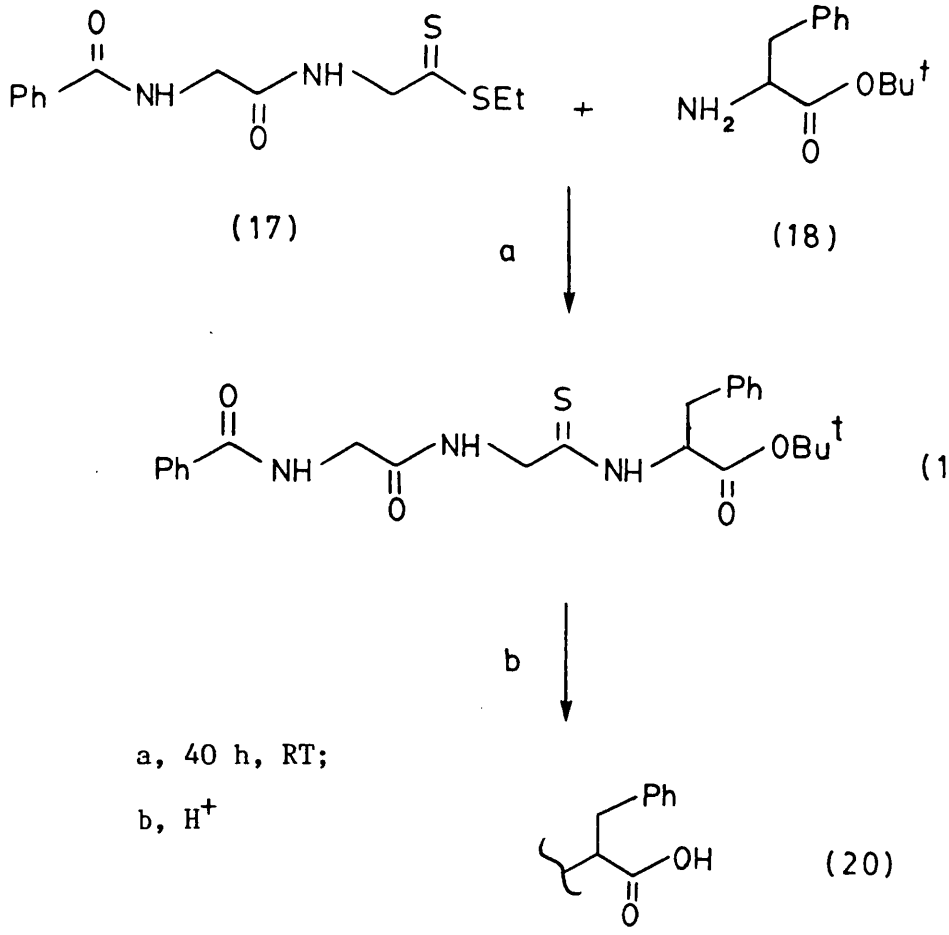
(12)



(13)

a, $Et_3\overset{+}{O}\ BF_4^-$, Δ, DCM, 0·75 h; b, TEA; c, H_2S, 8 h; d, TEA, amino acid, RT, 24 h.

Scheme 6

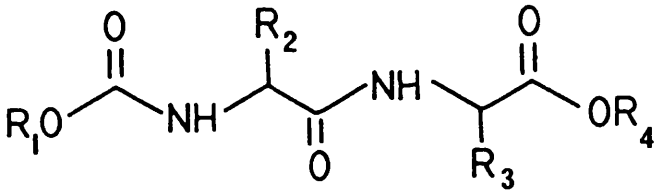


Although experimental details are brief one can envisage several steps in order to obtain (17)[41] and the coupling reaction requires a lengthy reaction time. The tripeptide free acid (20) was found to be much more slowly hydrolysed (over 1000 times), than its oxygen analogue, by Carboxypeptidase A.[39,40]

In the last few months other authors[42] have in fact extended the dithioester approach to the synthesis of the four possible modified enkephalins. Other endothiopeptides[43] have also been synthesized by this method and direct thionation methods. However, this work will be discussed more fully later (Results and Discussion).

1.4.C. Thionation Methods

Thus, it would be desirable to devise a short (preferably one- or two-step) route to endothiopeptides. The logical method involves direct thionation of N-protected dipeptide esters (21).



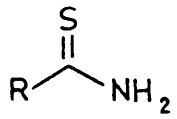
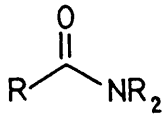
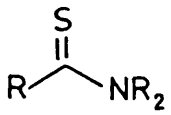
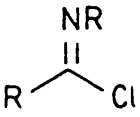
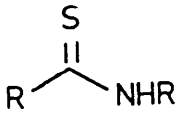
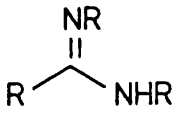
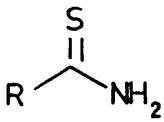
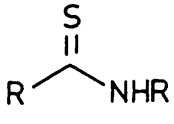
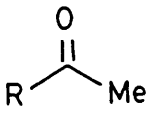
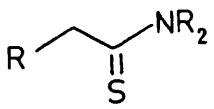
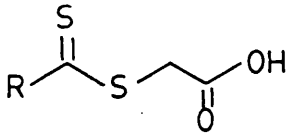
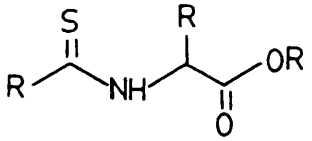
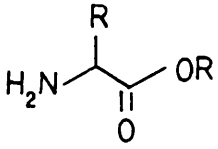
(21)

Once thionation of the amide bond has occurred deprotection at the amino or carboxyl terminus should allow access to larger systems.

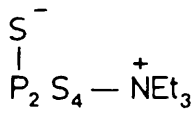
Although methods of preparation of thioamides are well known (Table 3)[31] direct thionation via phosphorus pentasulphide usually involves an excess of the reagent, prolonged reaction times and/or elevated temperatures. Additionally there is the problem of regio-selectivity in substrates of type (21). High temperature reaction conditions involving an excess of phosphorus pentasulphide could lead to polythionation of the carbamate, amide and ester carbonyl functionalities in (21) and presumably for some of these reasons the direct thionation method was reported as unsuccessful.[35] Other methods outlined in Table 3 include the problems associated with the work of Ried *et al.*;[34,35,38] i.e. several steps and rather drastic conditions.

Recently, however, several milder methods of thionation have been developed with phosphorus pentasulphide,[44,45] using ultrasonication to improve the homogeneity of the reaction and hence lower

Table 3 General methods of Thioamide formation[31] (pre 1978)

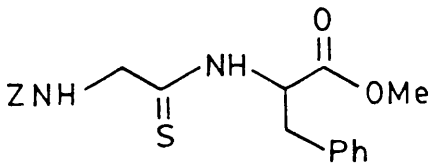
Starting material(s)		Product(s)
	H_2S, TEA Δ or P	
	P_2S_5, Δ	
	H_2S	
	H_2S	 or 
	S_8, RNH_2 Δ	
	Py	
		

reaction times and temperatures[44] and also, in combination with triethylamine and a polar solvent such as acetonitrile where a charged species (22) is possibly involved.[45]



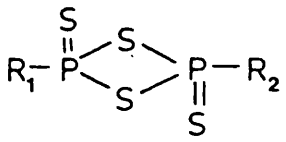
(22)

Additionally, a novel mild thionation method has been successfully used on simple substrates with the *in situ* formation of boron trisulphide (B_2S_3) from Bis(tricyclohexyltin)sulphide and boron trichloride.[46] This method is claimed to operate under neutral conditions although the use of boron trichloride may cause problems with substrates of type (21) where the amino and carboxyl protecting groups are Lewis acid sensitive (e.g. tert-butoxycarbonyl). A facile way round this problem would be to preform the boron trisulphide and then add the substrate. These methods deserve further investigation applied to peptide substrates although the phosphorus pentasulphide/triethylamine system has been used to obtain the single endothiodipeptide (23).[47] This was deprotected at the carboxyl end via alkali treatment to give the free acid which was a mild, competitive inhibitor of Carboxypeptidase A. Although the endothiodipeptide bound to the enzyme at a comparable rate to its oxygen analogue it was hydrolysed at only 10% of the rate. This may reflect the degree to which the enzyme-substrate tetrahedral transition state is *irreversibly* bound. This however was the only example studied.



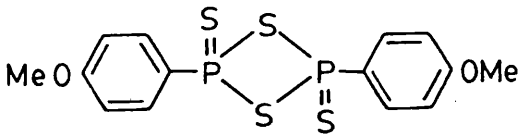
(23)

More recently, phosphetane disulphides (24)[48] have been utilized as thionation agents.



(24)

The conversion of carbonyl compounds to their thiono-derivatives using 2,4-bis(4'-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulphide, (25) (Lawesson's reagent) proceeds in high yields[49,50] and specifically

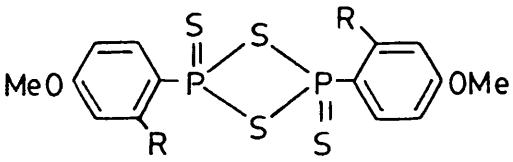


(25)

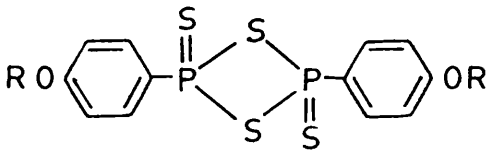
thioamides have been prepared from amides.[51,52,53] Prior to the beginning of this programme (25) had not been used for the thionation of peptide substrates.[54] The efficiency of the reagent in other systems warrants its application to suitably protected dipeptide substrates ((25) is known to react with the amino and carboxyl functionalities[51]). Regioselectivity of thionation was unknown (amide vs carbamate vs ester carbonyl) although based on literature precedent[50] the ester carbonyl was expected to be the least reactive.

Further, more subtle regiochemical control where several amide bonds are present, in higher peptides had not been explored. To what extent does the amino acid side chain (R_2,R_3 in (21)) influence the thionation? Modification of the original reagent, as follows in (26)

and (27), may allow greater reactivity and/or selectivity, especially in (26) where steric crowding may allow this reagent to differentiate between amide bonds. It would be desirable to selectively thionate a specific amide link in higher peptides which would give rapid access to larger systems with one thioamide bond present.[55] The latter approach may have severe limitations, however, where a Gly-Gly



(26)



(27)

R≠ Me

27a, R= Ph

dipeptide link is present if it is desired to thionate a different, more crowded, amide bond in the molecule. Being much more sterically less hindered, the Gly-Gly bond will probably always thionate in such a case.

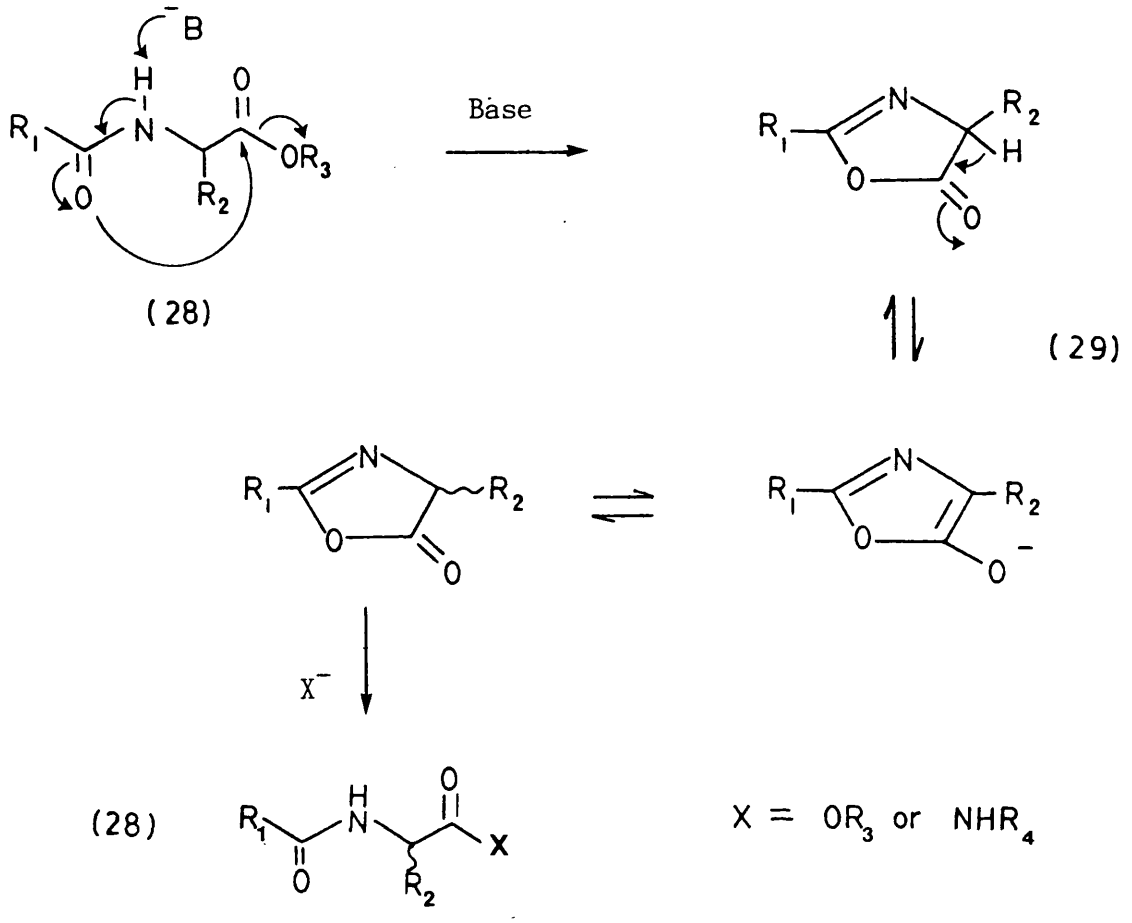
1.4.D. Racemization

It is evident that more conventional peptide chemistry (coupling, protection, deprotection methods) will have to be used to surmount the latter problem. To what extent are these techniques applicable to the new systems? Racemization is also a key issue in peptide chemistry and the effect of (25) on the stereochemical integrity of amino acid chiral

centres was unknown. If thionation occurs selectively at the amide bond will the more nucleophilic sulphur atom cause scrambling (racemization) of the stereocentres in subsequent deprotection and coupling steps?

A cause of racemization in conventional peptide chemistry is the formation of 2-oxazol-5-ones (29) from active esters of acylamino acids (28)[56] (Scheme 7).

Scheme 7



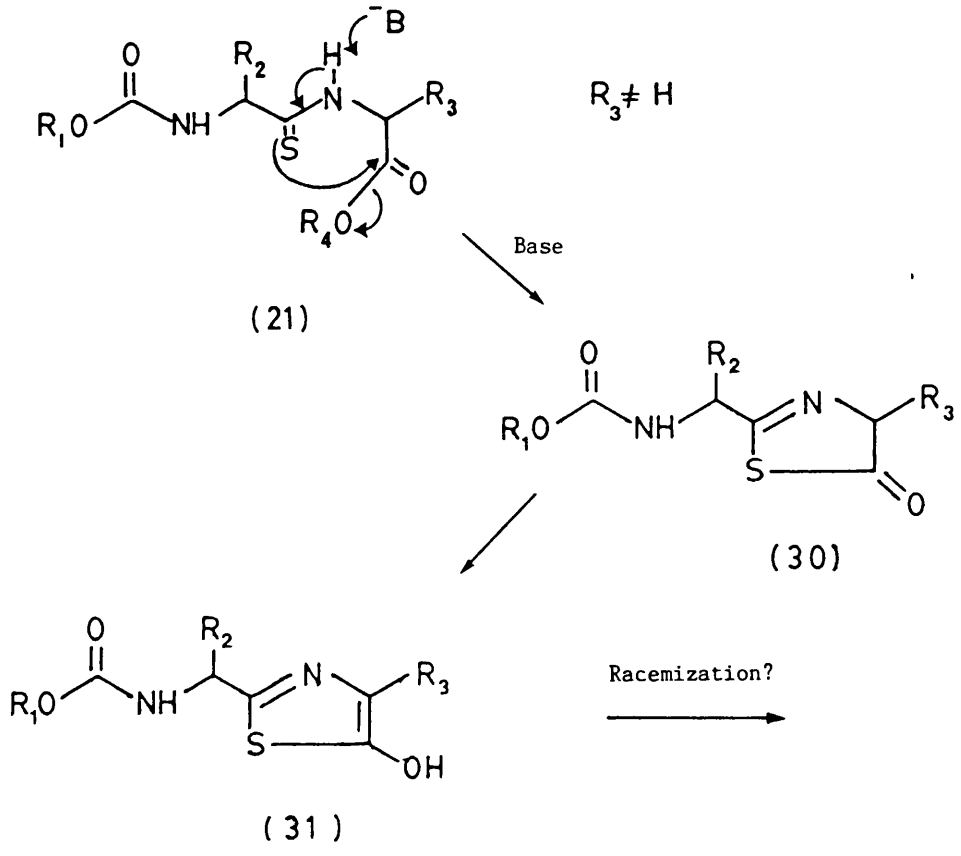
The presence of base in the reaction mixture causes proton abstraction and a "5-exo-trig" intramolecular ring closure with loss of the active ester species. Enolization of the oxazolone causes racemization once the ring (29) is reopened by alkoxide or by the free amino component

present in the reaction. The resultant dipeptide has racemized.

This problem is usually circumvented firstly by the use of alkyloxycarbonyl amine protective groups, which do not form oxazolones as readily and for which the corresponding alkyloxyoxazolones have a far less acidic proton than their alkyl counterparts (from acylamino peptides),[57] or secondly by the use of reaction conditions that do not accentuate oxazolone formation[56] (i.e. base equivalence, low temperatures, low polarity solvents).

For the thioamide analogues a similar problem may arise in *dipeptide* substrates (21) by the formation of 2-thiazol-5-ones, (30), (Scheme 8) and their tautomerism with the "enol" (31).

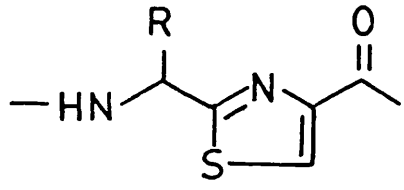
Scheme 8



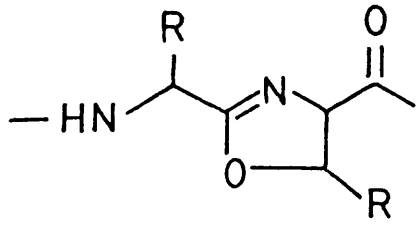
The extent of this potential problem in endothiopeptides is as yet unknown. 2-Thiazol-5-ones (30) formation can also be envisaged as occurring from the N-protected endothiodipeptide acids (13) via the dehydrative effect of DCC in coupling reactions.[58] This may alter the rate of reaction dramatically to adverse effect.

1.5. ENDOTHIOAZIRIDINE PEPTIDES

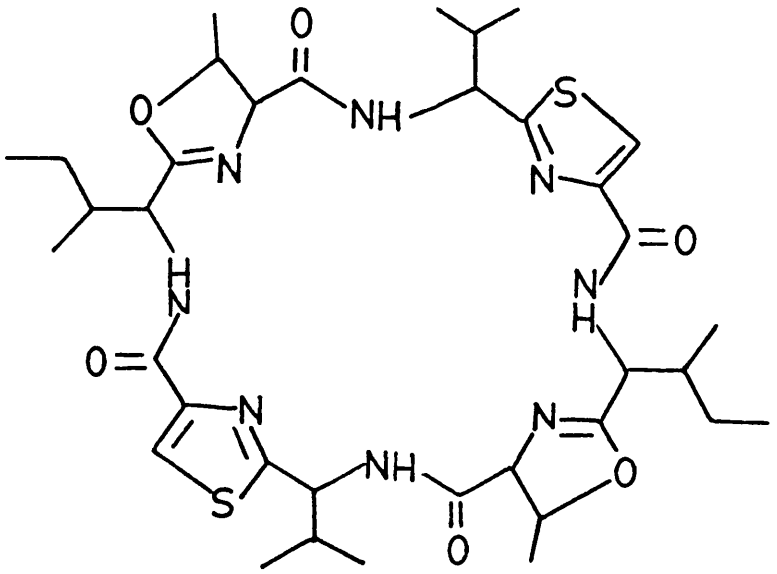
The reactions of endothiopeptides may allow novel and efficient access into other biologically interesting targets. Recently, a number of cyclic peptides with active anti-microbial and neurophysiological properties have been isolated from marine sources.[59-62] Many of these contain the thiazole amino acid unit (32) in an alternating arrangement either with other amino acids or with 2-oxazoline acids (33).



(32)



(33)

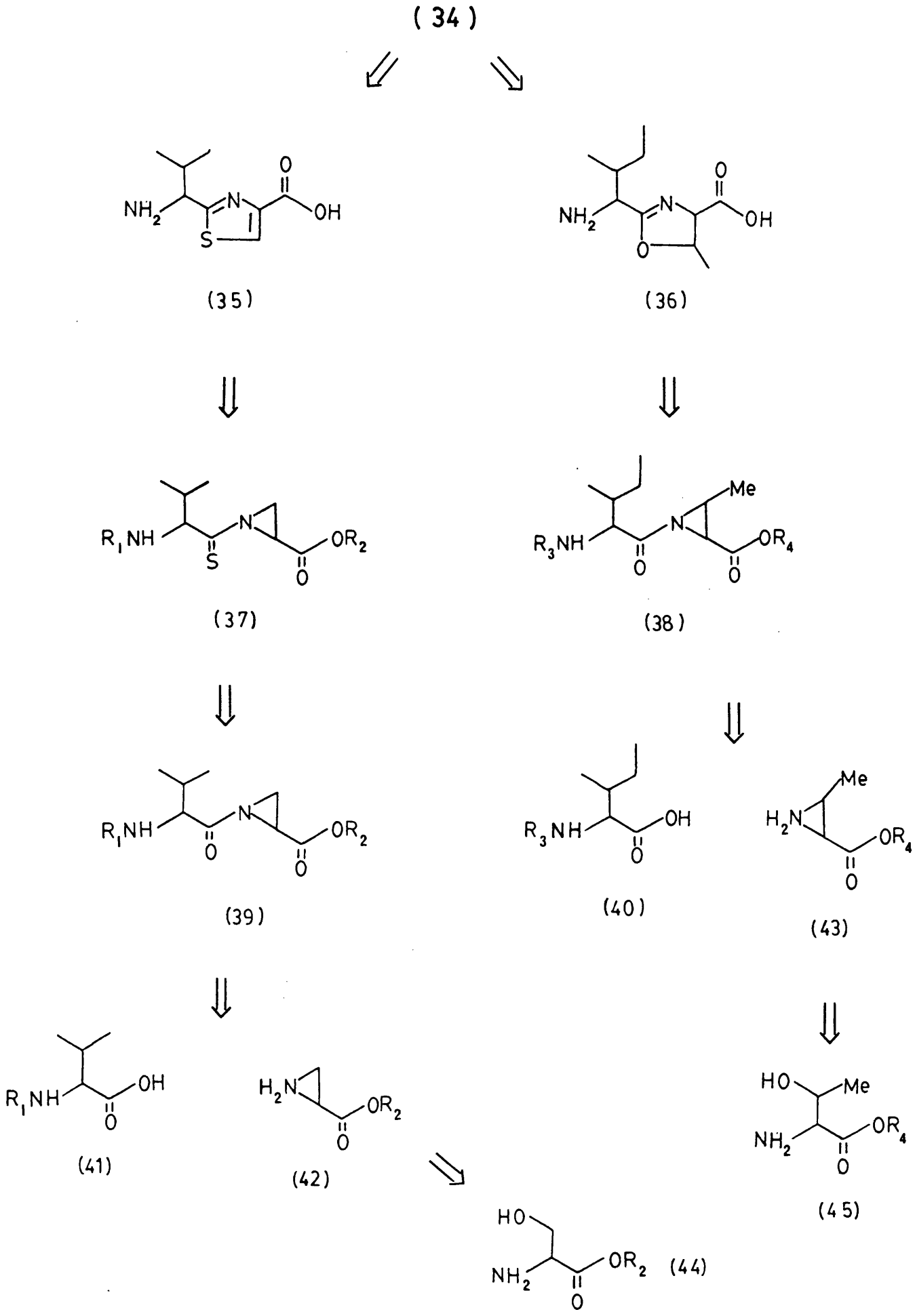


(34)

Ascidiacyclamide, (34)[63] contains both these units and is a potent cytotoxic agent. Its synthesis and that of related analogues may produce pharmacologically useful molecules. A retrosynthetic scheme directed at this target is shown in Scheme 9.

The symmetrical nature of (34) means that it can be analysed into two dipeptide derived units (35) and (36) containing the thiazole and 2-oxazoline moieties. The former may be envisaged as being obtained (via an aziridine isomerization, then oxidation) from the endothio-aziridine peptide (37). The latter systems are not known in the literature although the isomerization of acyl and thioacyl aziridines

Scheme 9

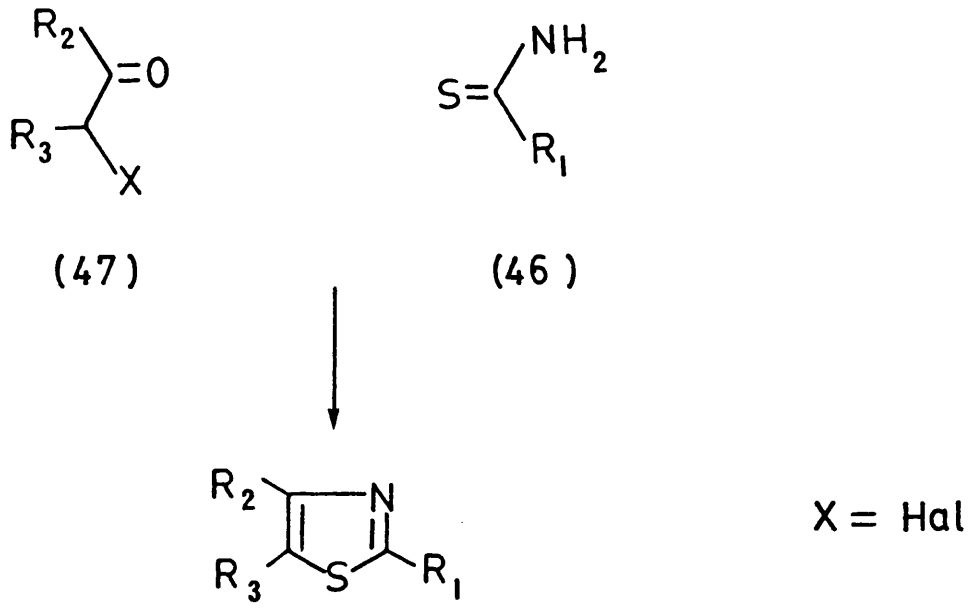


has been investigated[64] and reviewed.[65] The use of peptide systems like (37) for the preparation of thiazoles has not been explored although the attempted isomerization of aziridine containing peptides similar to (38) has been reported[66] using sodium iodide. Other methods for the conversion have not been tried but it is expected that (37) should undergo this isomerization more easily[65] than (38).

Both (37) and (38) are derivable from simple amino acids although the reactivity of aziridines towards thionation agents like (25) is not known. Other milder thionation methods (see previous discussion) may be necessary to convert (39) to the thioaziridine (37). The N-protected amino acids *iso*-leucine (40) and valine (41) are readily available. Suitable serine and threonine esters ((44) and (45)) are known to give the desired aziridines (42) and (43) although (42) is not well described in the relevant literature.[67,68] Both aziridines have been used by Japanese workers to obtain other peptide derivatives through stereo-controlled aziridine formation and ring opening (two inversions, net retention of configuration). For example, depsi-peptides,[69] dehydroamino acids[70] and cysteine peptides[71] have been derived from aziridines.

Previously more conventional routes into thiazoles and thiazole amino acids, including (35), have been reported.[72] The Hantzsch synthesis[73] involving the condensation of a thioamide (46) with an α-haloketone, (47), has been extensively used in thiazole (48) synthesis[73] (Scheme 10).

Scheme 10

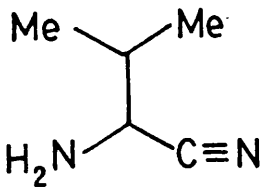


(47) (46)

(48)

In this manner (35) has been prepared[72] in 6 steps via the Strecker synthesis[36] of the appropriate α-amino nitrile (49).

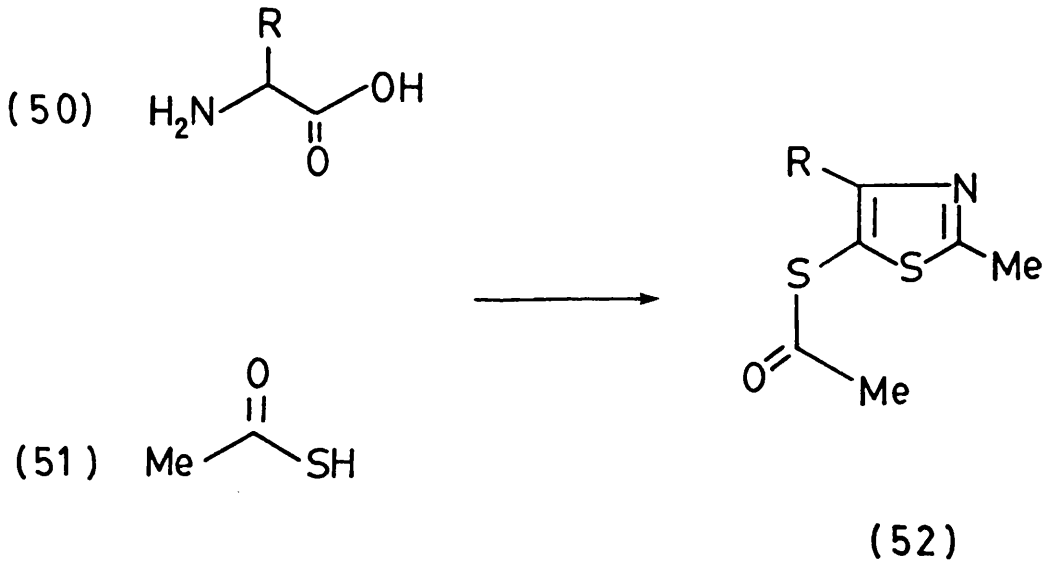
(49)



No control of chirality is possible in this route because of the nature of the Strecker reaction.

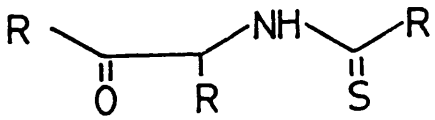
Thiazoles (52) have also been obtained from amino acids (50) and thiolacetic acids (51) (Scheme 11)[73] and by the intramolecular

Scheme 11



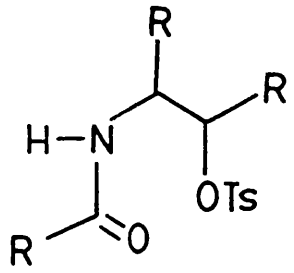
cyclization of α-thioacylamino ketones (53).[73]

(53)



However, the proposed route has the advantage of being novel and exploring the hitherto unknown chemistry of endothioaziridine peptides.

2-Oxazolines (for example (36)) are generally prepared via a suitable acyl-β-amino alcohol derivative like the tosylate (54).[74]



(54)

To make the route to Ascidiacylamide (34) strategically more convergent the isomerization of aziridine peptides to 2-oxazolines (so far unknown) is preferred.

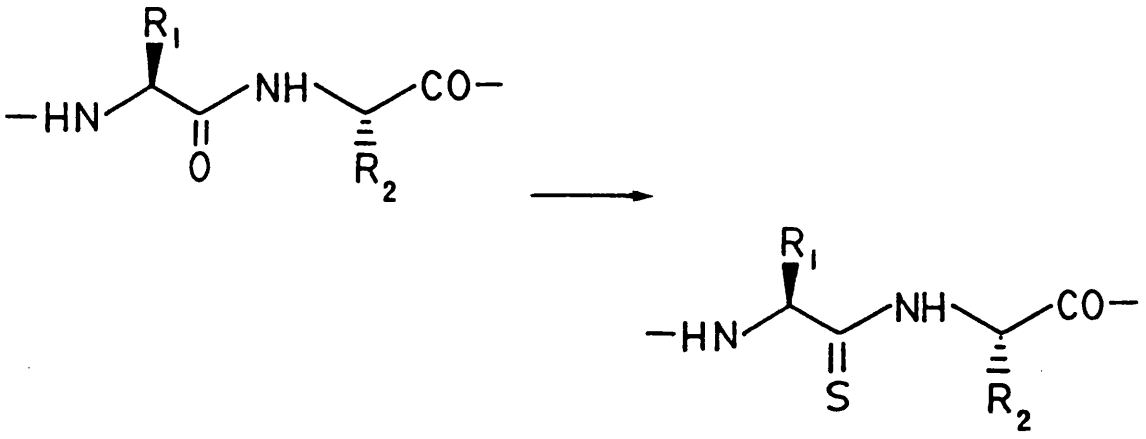
1.6. CONCLUSION

The synthesis of endothiopeptides has not been fully explored and their biochemistry and pharmacological properties not investigated. Modifications of biologically important peptides (such as the enkephalins) with the thioamide functionality offer great potential. A study of these systems may answer some questions, and raise others, about enzyme-substrate interactions and mechanisms.

RESULTS AND DISCUSSION

2.1. THIONATION OF DIPEPTIDES

The initial purpose of this work was to develop a synthetic route(s) to peptides containing the thioamide functionality. Selective replacement of amide bonds in small peptides by their sulphur counterpart may have important biological considerations (see Introduction) i.e.

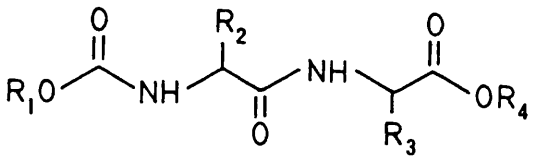


A direct route to endothiopeptide systems has obvious advantages and the use of Lawesson's reagent (25)[48] as a thionation agent was explored.[54,75] Unprotected dipeptides were not used as substrates since (25) has been shown to react with both amino and hydroxyl functionalities.[51] Additionally, the use of protected dipeptides should give greater flexibility because of the potential deprotection of *either* terminus after thionation. Thus, readily available N-alkyloxycarbonyl dipeptide esters (21) (Table 4) were thionated exclusively at the amide carbonyl in high yields (70-100%) using (25) (Scheme 12).

Table 4 : Substrates and products of the thionation reactions

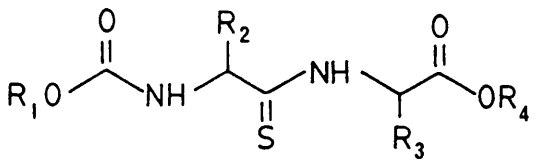
Substrate (Product)	R^1	R^2	R^3	R^4
21a (55a)	$PhCH_2$	H	H	CH_3
21b (55b)	$PhCH_2$	H	H	CH_2-C$_6$H$_4$-OCH_3
21c (55c)	$PhCH_2$	H	H	Ph
21d (55d)	Bu^t	H	H	CH_2Ph
21e (55e)	$PhCH_2$	CH_3	CH_3	CH_3
21f (55f)	$PhCH_2$	$PhCH_2$	H	CH_3
21g (55g)	$PhCH_2$	$CH_2CH(CH_3)_2$	H	CH_3
21h (23)	$PhCH_2$	H	CH_2Ph	CH_3

Scheme 12



(21)

(25)



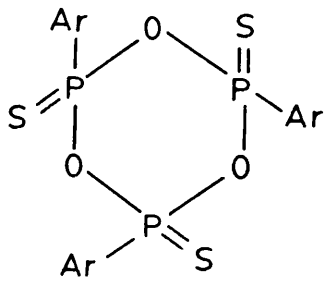
(55 a-g)

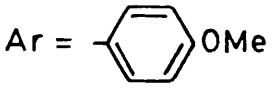
(23)

No thionation was observed at the ester or carbamate carbonyl groups.[54,75] A summary of the endothiodipeptides ((55a-g) and (23))

obtained is shown in Table 5. The products are rapidly formed and easily purified by column chromatography. A side product (56) from the thionation[51,54a] is easily separated from the desired products.

(56)





The spectral data of the endothiopeptides ((55) and (23)) show obvious similarities with the starting materials (21). The ^{13}C n.m.r. spectra, however, show that the amide carbon is shifted downfield by about 30 ppm to ~200 ppm. The ester and carbamate carbonyl signals remain the same. The methylene and methine carbons of the peptide backbone are also shifted downfield, by between 5 and 8 ppm, compared to the normal dipeptides.

The most notable difference in the ^{1}H n.m.r. spectra is the characteristic downfield shift (1·5–2·0 ppm) of the NH proton of the newly formed thioamide group. The carbamate NH proton is less affected by the transformation and the methylene and methine protons of the backbone are generally more shielded (0·25–0·4 ppm) than their oxygen counterparts.

Table 5 : Experimental details for preparation of N-protected endothiodipeptide esters ((55) and (23))[a]

Product	Temperature (°C)	Time (h)	Column solvent[b]	Yield (%)	mp (°C)
Z-Glyt-Gly-OMe (55a)	100	2·5	10	92	89-90[d]
Z-Glyt-Gly-OmBzl (55b)	85-95	0·7	7·5	80	105-106
Boc-Glyt-Gly-OBzl (55d)	85-95	2	10	89	103·5-105[d]
Z-Phet-Gly-OMe (55f)	85-90	2·5	10	83	68-69·5[d]
Z-Ilet-Gly-OMe (55g)	85-90	1·8	10	86	-[c]
Z-Glyt-Phe-OMe (23)	80-90	0·7	5	94	-[c]

a With the exception of (55c) and (55e) (described in 'Experimental')

b % EtOAc/DCM

c Obtained as colourless gums

d Recrystallized from MeOH/water (50:50)

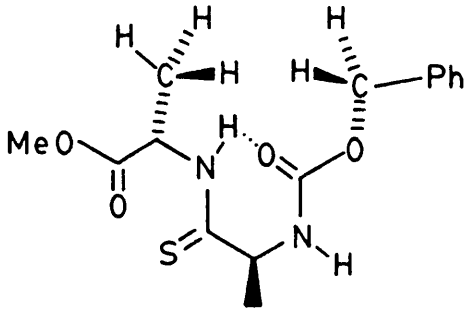
The U.V. and I.R. spectra are also indicative of the thioamide functionality. The amide carbonyl stretching frequency at 1650 cm^{-1} completely disappears in the I.R. spectra of (55) and (23) while the U.V. spectra show a maximum absorption at 260-270 nm, characteristic of a $\Pi \rightarrow \Pi^*$ transition for the thiocarbonyl group.[31,54a]

To check that no racemization of the adjacent chiral centres had occurred in the thionation, a 400 MHz ^1H n.m.r. spectrum of (S,S)-Z-Alat-Ala-OMe (55e) was obtained. The latter shows overlapping doublets (1·46 and 1·47 ppm, J = 7 Hz) of the methyl groups and pentets (4·6 and 5·05 ppm, $J_{H-H} = J_{NH-H} = 7$ Hz) for the methine protons. The high signal to noise ratio and sensitivity of the technique would allow detection of other diastereoisomers of >2% but no other signals were seen.

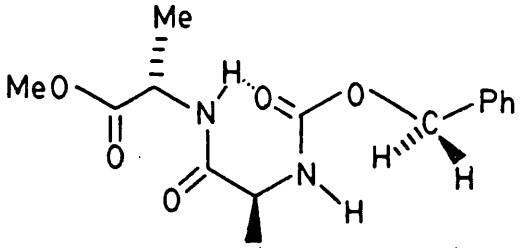
The conformational consequences of the introduction of the thioamide group were studied by differential n.O.e.[76] spectra of (21e) and (55e). Each compound was similar in showing effects consistent with the favoured conformation(s) ((57) and (58)) where the amide (or thioamide) NH is hydrogen bonded to the carbamate carbonyl oxygen atom. For (55e) irradiation of the Ala^2 methyl group produced an enhancement (33%) of the methylene proton signal which was non-existent in (21e). However, the latter showed instead an enhancement (14%) of the carbamate NH proton signal when the methylene group was irradiated. These results are consistent with the favoured conformations shown ((57) and (58)).[75]

The thioamide proton is more acidic than the amide case which could lead to stronger hydrogen bonding in (57). The resulting depletion in electron density in the carbamate carbonyl may result in its C-O bond having more double bond character favouring the different conformation (58) about the carbamate C-O bond.

(57)



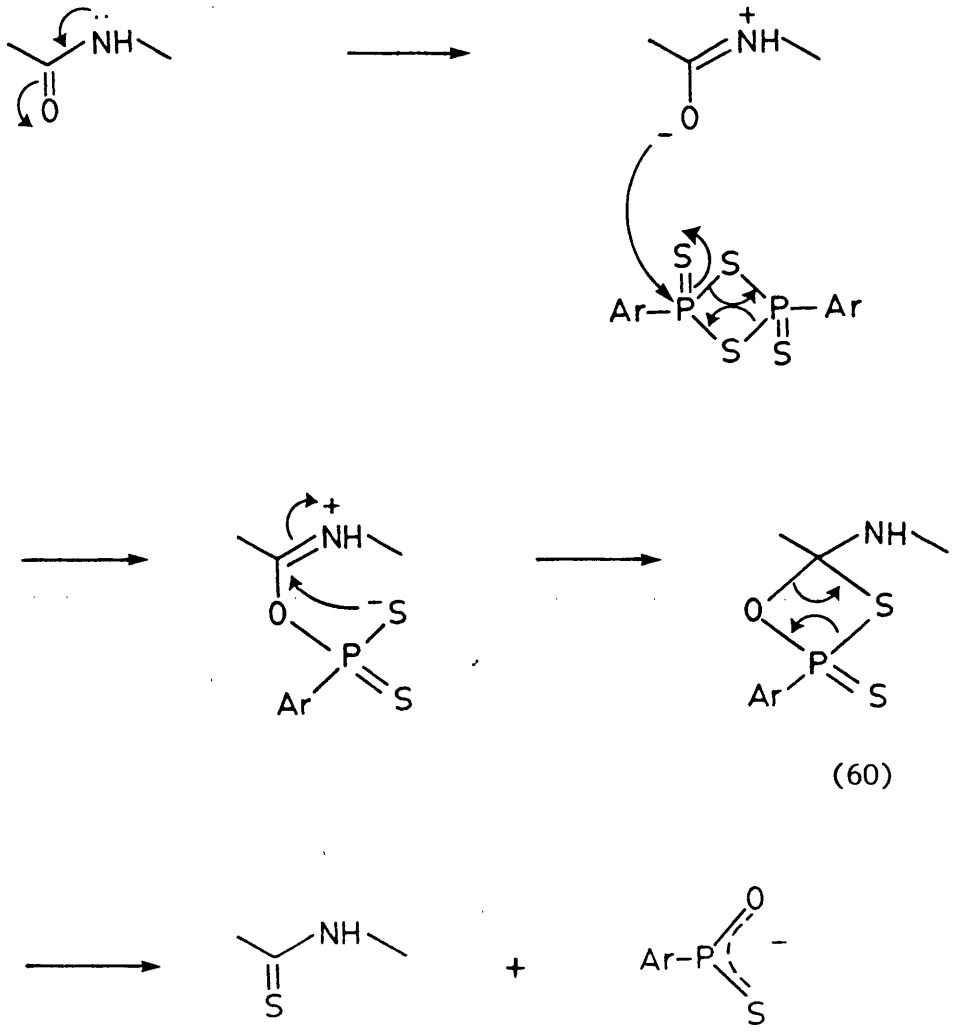
(58)



The mechanism drawn for the thionation reaction is speculative at this point. A possibility is shown below (Scheme 13) and may involve a cyclic intermediate (60) which decomposes, the driving force being P–O bond formation.

Differences in the rate of thionation, for example between (21a) and (21g) were not observed. Although longer reaction times were used in some cases, most or all of the desired product was rapidly formed (within 1 h). Thus it seems unlikely that higher peptides could be selectively thionated at a specific *amide* link using Lawesson's reagent (25), although the possibility of using modified reagents (for example (26) and (27)) in this fashion is not precluded.[55] The use of moderate temperatures in this reaction is only necessary to achieve homogeneity since Lawesson's reagent (25) is not readily soluble in most organic solvents at room temperature. It is possible that the thionation

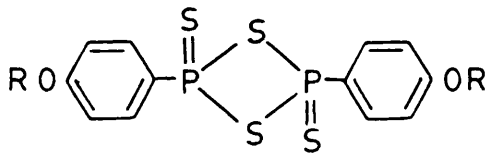
Scheme 13



(60)

could be carried out at lower temperatures if a better solvent could be found. This may itself lead to greater selectivity.

Recent work by Lajoie *et al.*[55] has illustrated the possibility by using the modified reagent (27a). The greater solubility shown by (27a) allows the thionation to be carried out at room temperature or below and greater selectivity is achieved. Thionation of the least sterically hindered amide link is preferred. For example, glycine-glycine amide bonds are thionated preferentially in oligopeptides.

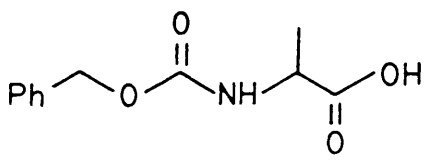


27a, R= Ph

However, this is a disadvantage in systems where the desired thioamide link is the *more crowded* one. In such cases conventional peptide coupling, protection and deprotection techniques[77] may be required to incorporate the thioamide functionality, via a stepwise or fragment coupling approach using simple endothiodipeptide systems such as (55). A range of protective groups is compatible with the thionation procedure (Table 5) and their selective removal was next investigated.

2.2. DEPROTECTION METHODS

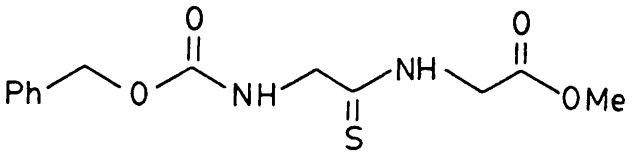
It was found that the endothiodipeptide Z-Glyt-Gly-OMe (55a) could be deprotected at the amino terminus using hydrogen bromide in acetic acid[78] at room temperature (<0·25 h) giving the hydrobromide salt (61) in excellent yield (95%) (Scheme 14). The feasibility of synthesizing higher peptides by extending from the amino end was confirmed by the coupling of Z-Ala-OH (62) with the hydrobromide salt (61) using DCC/TEA [79] giving the novel tripeptide Z-Ala-Glyt-Gly-OMe (63).



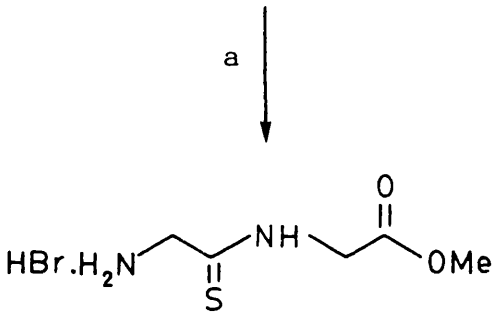
(62)

This represents the specific introduction of a thioamide group in a tripeptide.

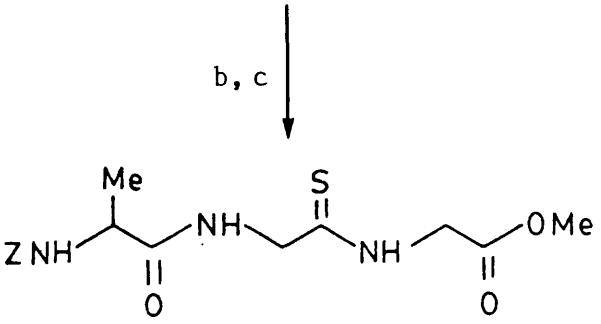
Scheme 14



(55a)



(61)

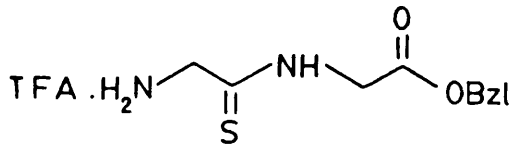


(63)

a, HBr/AcOH, RT, 0·25 h; b, (62), DCC, -20°C, 0·25 h; c, (61), TEA, RT, 16 h.

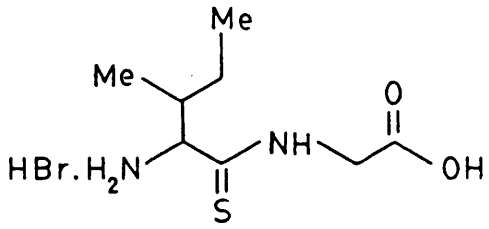
A slightly different acidolytic cleavage procedure for removal of the *tert*-butoxycarbonyl amino protective group from Boc-Glyt-Gly-OBzl (55b) using TFA (90%)[80] gave the trifluoroacetate salt (64). The thioamide group in both these examples was untouched by acidic reagents. Other workers[33b] have reported that thioamides are not stable to TFA but in our case no problems were observed.

(64)



However, treatment of Z-Ilet-Gly-OMe (55g) with HBr/AcOH at room temperature (1·5 h) gave the free endothiodipeptide acid (65) under surprisingly mild conditions where both amino and carboxyl protective groups had been removed.

(65)

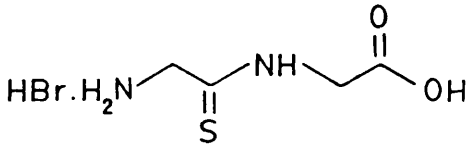


Methyl esters of N-protected peptides are normally not acid labile under these mild conditions.[81] Indeed the benzyloxycarbonyl (Z) amino protective group is commonly used because of its compatibility with, and selective cleavage from, the methyl ester group.[82] That the free acid had been obtained was conclusively demonstrated by its n.m.r. data. The ^{1}H spectrum of (65) showed a singlet at 4·45 ppm and a doublet at 4·05 ppm attributed to the methylene and methine protons (no coupling with NH because of proton exchange with solvent) of the peptide backbone. The methyl ester protons would be expected to resonate in the 3·5–4·0 ppm region but no signals were observed in this part of the spectrum. Other signals were as expected for an *iso*-leucyl containing peptide. The ^{13}C n.m.r. spectrum showed signals

similar in chemical shift to starting material (55g) with two important exceptions. There was no methyl ester carbon signal at ~52 ppm and the Z group signals had also disappeared. The thioamide carbon was present at 200·9 ppm.

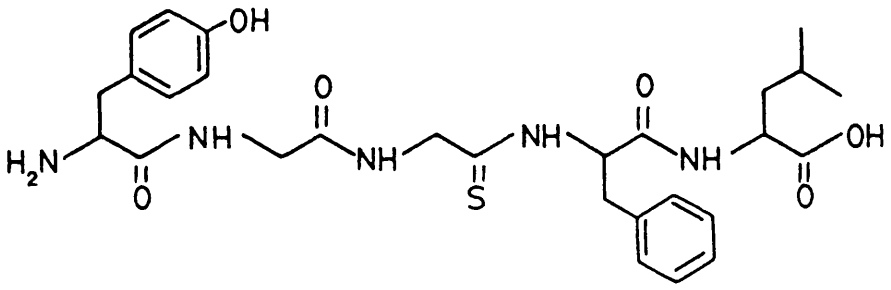
It is difficult to account for the unexpected sensitivity of this dipeptide (55g) towards acidic conditions. The complete deprotection of Z-Glyt-Gly-OMe (55a) by HBr/AcOH required both a longer reaction time (6 h) and a higher temperature (50-60°C) to remove amino and carboxyl protective groups. The reaction gave a precipitate of thioglycyl-glycine hydrobromide salt (66) when ether was added to the cooled reaction mixture.

(66)



The I.R. spectrum of the product (66) showed a broad OH stretch at 2900 cm^{-1} and the disappearance of any carbonyl stretching frequencies above 1650 cm^{-1}. A rather weak band was seen at 1620 cm^{-1} attributable to the carboxylate functionality. The ^1H n.m.r. spectrum showed the two methylene glycine protons as two singlets at 3·9 and 4·1 ppm (all other protons exchanged with solvent). The mass spectrum of (66) gave a weak molecular ion at m/e 148 (M-HBr) and characteristic peaks at 130 (148-H_2O) and 114 (130-NH_2).

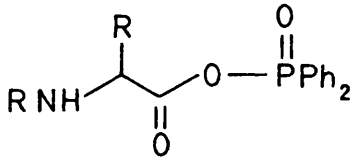
The endothiodipeptide Z-Glyt-Phe-OMe (23) is a possible precursor of the thionated analogue, H-Tyr-Gly-Glyt-Phe-Leu-OH (67) of the opioid pentapeptide leucine enkephalin.



(67)

It may be more active *in vivo* than the natural peptide because of its greater enzyme resistance. Conventional peptide techniques should allow the synthesis of this compound, starting with deprotection at either end of the dipeptide (23). Cleavage of the benzyloxycarbonyl (Z) amino protective group gave the hydrobromide salt (68) (79%). The latter was coupled to Z-Gly-OH (69) using a newly developed coupling agent[83] (diphenylphosphinyl chloride, DPPCl (70)) (Scheme 15).

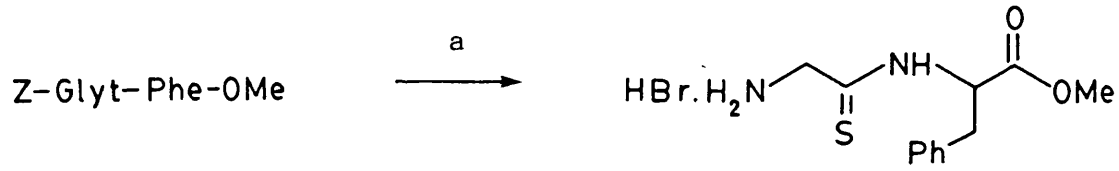
DPPCl (70) rapidly forms a carboxylic-phosphinic mixed anhydride (72) with N-protected amino acids which is then attacked, regioselectively at carbon, by nitrogen nucleophiles forming the amide bond.[83]



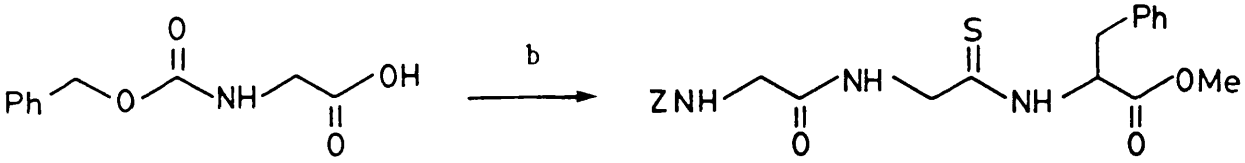
(72)

The side product from the reaction is diphenylphosphinic acid (73) which is easily removed by aqueous washing. This gave the endothiotripeptide, Z-Gly-Glyt-Phe-OMe (71), the free acid of which could itself

Scheme 15



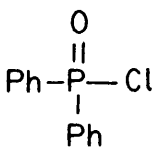
(23) (68)



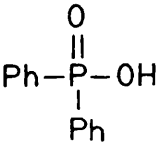
(69) (71)

a, HBr/AcOH, RT, 0·5 h; b, (70), NMM, -20°C, 0·25 h then (68), NMM, 16 h to RT.

be an inhibitor of carboxypeptidase (enkephalinase) enzymes.[39,40]



(70)



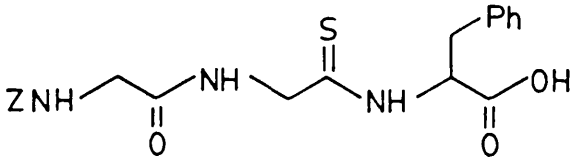
(73)

However, our attempts to deprotect the methyl ester by alkaline hydrolysis of the N-protected endothiopeptide esters (55) were only partly successful.

For instance, treatment of Z-Glyt-Gly-OMe (55a) with an equivalent of sodium hydroxide overnight[84] gave a deeply coloured (orange) solution which after a standard work-up gave the free acid (13a) (Scheme 16) (55%) (^{1}H n.m.r., MS) in an impure form.

A possible explanation for the moderate yield of product may be the formation of the sodium thioimidate (74).[85] This may then further react intramolecularly to form the 2-thiazol-5-one (75⇌76) with expulsion of methoxide ion. However, the latter compounds were not isolated from the reaction mixture.

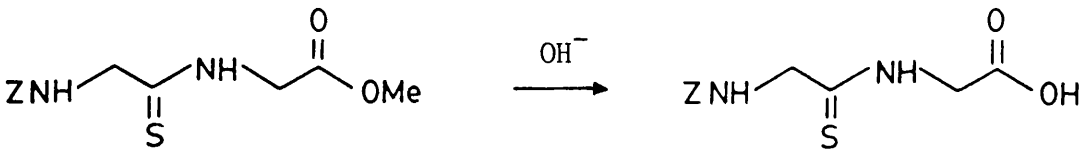
Treatment of (71) in a similar manner gave, after an acidic work-up, a two component mixture which was separated by chromatography giving the free acid (77) (60 MHz ^{1}H n.m.r., TLC) (60% yield).



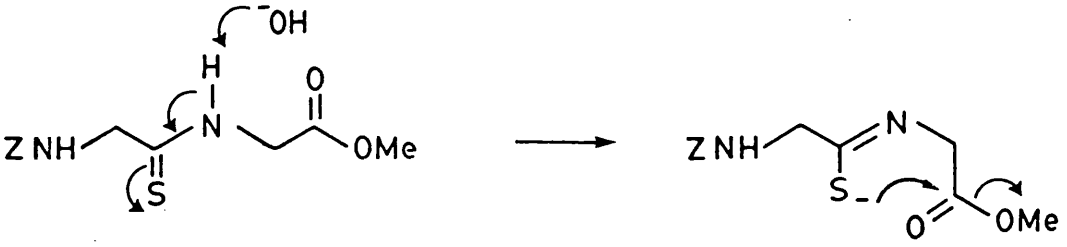
(77)

A trial experiment on a small amount of (71) using HBr/AcOH to effect deprotection of the benzyloxycarbonyl protective group gave what appeared to be the doubly deprotected tripeptide (78) instead of the free amino compound (79) (60 MHz ^{1}H n.m.r., TLC).

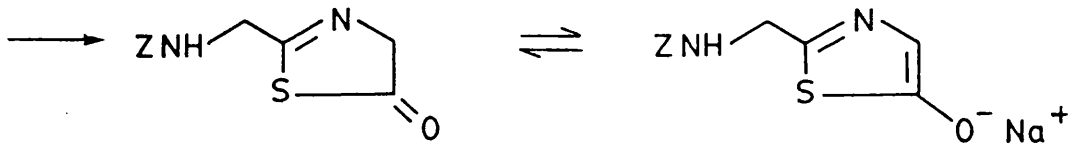
Scheme 16



(55a) (13a)

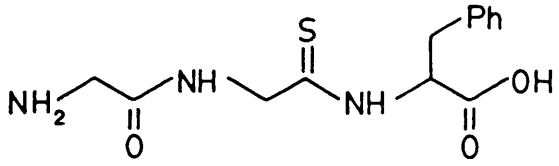


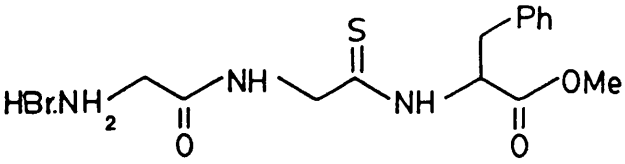
(74)



(75) (76)

The enhanced reactivity of (71) towards acidic reagents is difficult to explain but has been also observed with Z-Ilet-Gly-OMe (55g). In view of these only partly successful results and because of the more promising work described in the following section, further

(78) 

(79) 

work on the tripeptide, Z-Gly-Glyt-Phe-OMe (71) was curtailed. Although methods of carboxyl deprotection via alkaline hydrolysis of N-protected endothiopeptide esters were possible, other carboxyl protective groups were investigated.

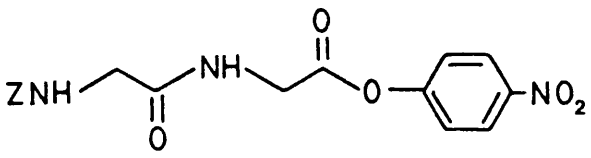
2.3. USE OF THE PHENYL ESTER PROTECTING GROUP

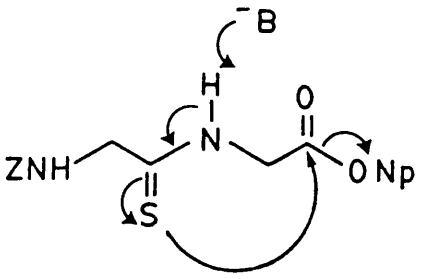
Ideally, an active ester group[86] would allow coupling at the carboxyl end without prior deprotection. The 4-nitrophenyl ester group has been used in this fashion.[87] However, an attempt to thionate Z-Gly-Gly- ONp (80)[88] using Lawesson's reagent (25) was unsuccessful (Scheme 17).

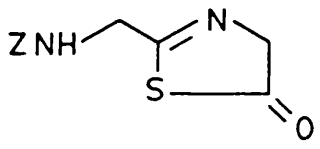
This reaction afforded several inseparable, unidentified products. Significantly, a small amount of 4-nitrophenol (82) was obtained from the reaction so that thionation possibly resulted in

Scheme 17

(80)







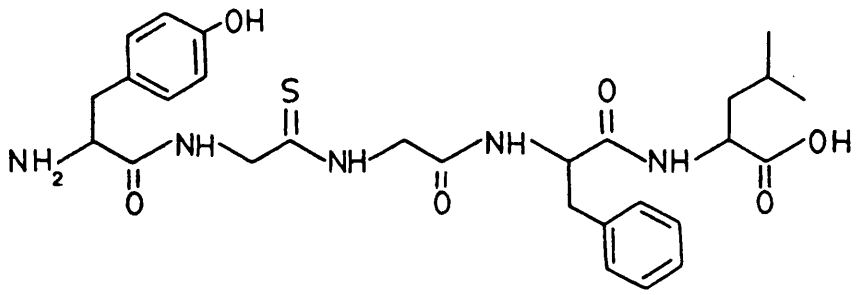
(75)

(82)

an intramolecular cyclization whereby the 4-nitrophenate anion was displaced by the thiono group (Scheme 17) giving the 2-thiazol-5-one (75). Although the latter compounds have been implicated in several reactions of endothiopeptides so far in this Thesis, their involvement has not been clearly demonstrated.

The use of the phenyl group of, for example, Z-Glyt-Gly-OPh (55c) as an active ester is also feasible.[89] The phenyl ester should be displaceable by an amino acid or dipeptide, albeit in a less facile

manner[90] than usual active esters. The dipeptide (55c) is thus a potential precursor of the leucine enkephalin analogue H-Tyr-Glyt-Gly-Phe-Leu-OH (83) (Scheme 19).

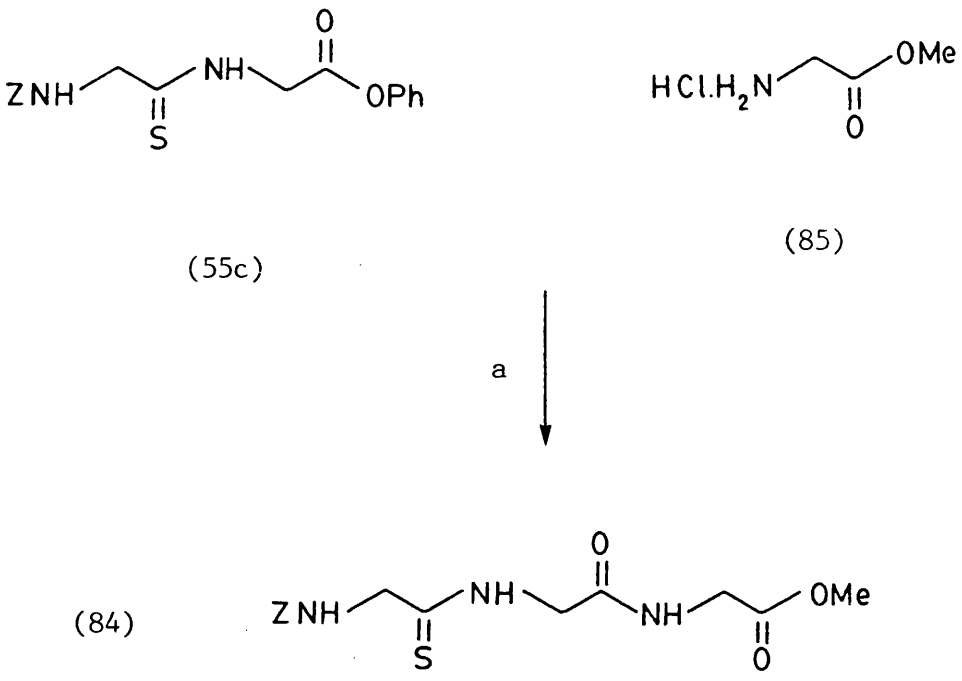


(87)

As a trial reaction to test this presumption, the tripeptide Z-Glyt-Gly-Gly-OMe (84) was prepared from (55c) and glycine methyl ester hydrochloride (85) (Scheme 18).

A refluxing solution (2-propanol) was required to effect the reaction in a short time. Displacement of phenol by the glycine residue gave the tripeptide (84) in moderate yield (47%). This represents the replacement of the *first* amide bond of a tripeptide by its sulphur analogue (cf (63) and (71)) where the second amide group was replaced). Although the yield is only moderate, the reaction is comparable with the two step sequence represented by alkaline hydrolysis of the dipeptide (55a) (55%) followed by coupling (using, for example, DPPCl) with glycine methyl ester (85) (which would be ~90%). Since the phenyl ester appeared to be a suitable, albeit fairly unreactive group, for our purposes the synthesis of (83) was undertaken.

Scheme 18



a, TEA, 2-propanol, Δ, 8·5 h.

2.4. SYNTHESIS OF H-Tyr-Glyt-Gly-Phe-Leu-OH (83)

2.4.A. [4+1] fragment synthesis of Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87)

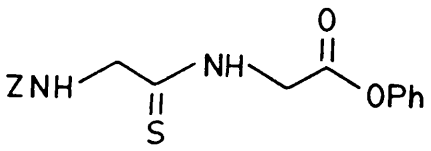
As previously mentioned, the dipeptide Z-Glyt-Gly-OPh (55c) is a suitable, readily available precursor of the target monothionated enkephalin (83).

Coupling of (55c) with the hydrochloride salt of the dipeptide H-Phe-Leu-OMe (88) was carried out following a similar method to that used for Z-Glyt-Gly-Gly-OMe (84) and gave the tetrapeptide Z-Glyt-Gly-Phe-Leu-OMe (86), albeit in slightly poorer than expected yield (43%) (Scheme 19).

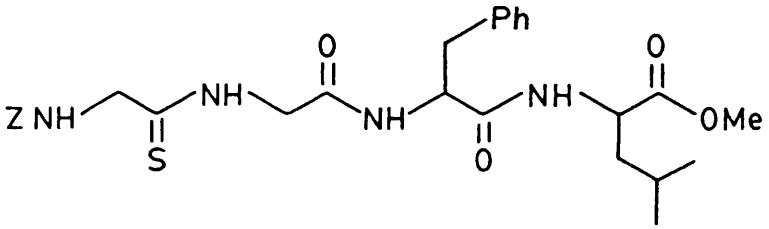
Scheme 19 : [4+1] route to Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87)

(21c) Z-Gly-Gly-OPh

a

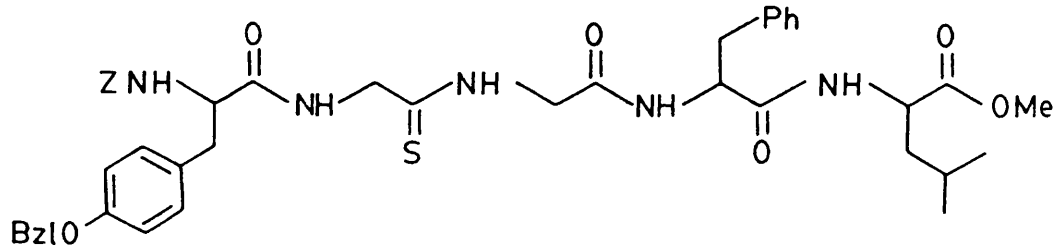
(55c) 

b



(86)

c, d

(87) 

a, (25), 80–90°C, 1 h; b, (88), TEA, 2-propanol, Δ, 2·5 h;

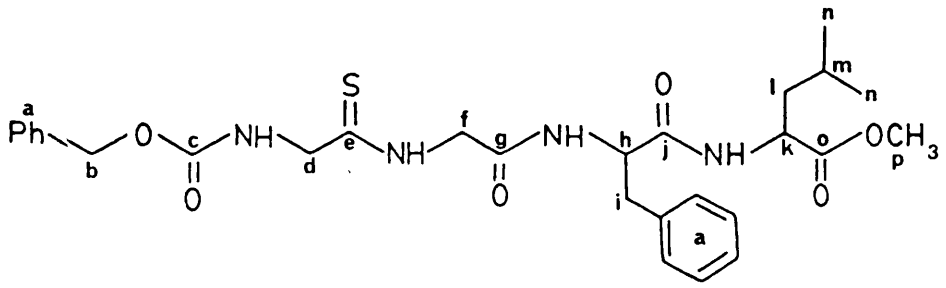
c, HBr/AcOH, RT, 0·5 h; d, (89), (70), NMM, -20°C, 0·5 h then (90), NMM, RT, 16 h.

H-Phe-Leu-OMe (88) Z-Tyr(Bzl)-OH (89)

All spectral data for (86) were in accord with its structure. The ^{1}H n.m.r. spectrum (400 MHz) showed signals indicative of a peptide containing phenylalanyl, leucyl and glycyl amino acid residues. In addition, a broad singlet (1H) was seen at 9·05 ppm, the characteristic low field value of the thioamide proton.

The ^{13}C n.m.r. spectrum (Figure 4) showed four carbonyl signals between 156 and 173 ppm due to the carbamate, amide (2) and ester groupings. The thioamide carbonyl appeared at 200·1 ppm consistent with previously described di- and tripeptides.[54,75]

Figure 4 : ^{13}C chemical shifts of (86)

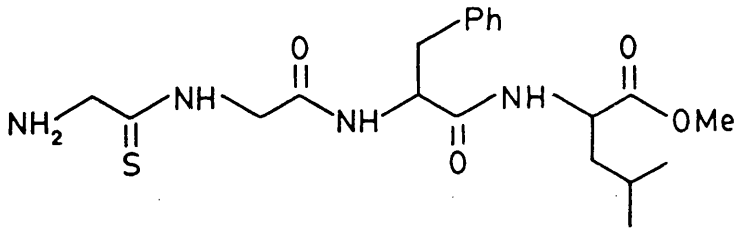


a	126·2–137·6	i	40·3
b	65·8	j	171 (172·6)
c	156·0	k	50·4 (51·3)
d	51·7	l	40·3
e	200·1	m	24·2
f	47·6	n	21·3 and 22·7
g	172·6 (171·0)	o	166·7
h	51·3 (50·4)	p	53·7

The FAB (+) mass spectrum of (86) showed the protonated molecular ion (m/e 557) with intense signals at 293 (Phe-Leu-OMe fragment), 146 (Leu-OMe) and 120 ($H_2\overset{+}{N}=CHCH_2Ph$).

Deprotection of the tetrapeptide (86) by HBr/AcOH at room temperature (0·5 h) gave, after column chromatography, an almost colourless oil, which was, tentatively (at this stage), identified (^1H n.m.r., MS) as the free amino compound (90), in a moderate yield (47%).

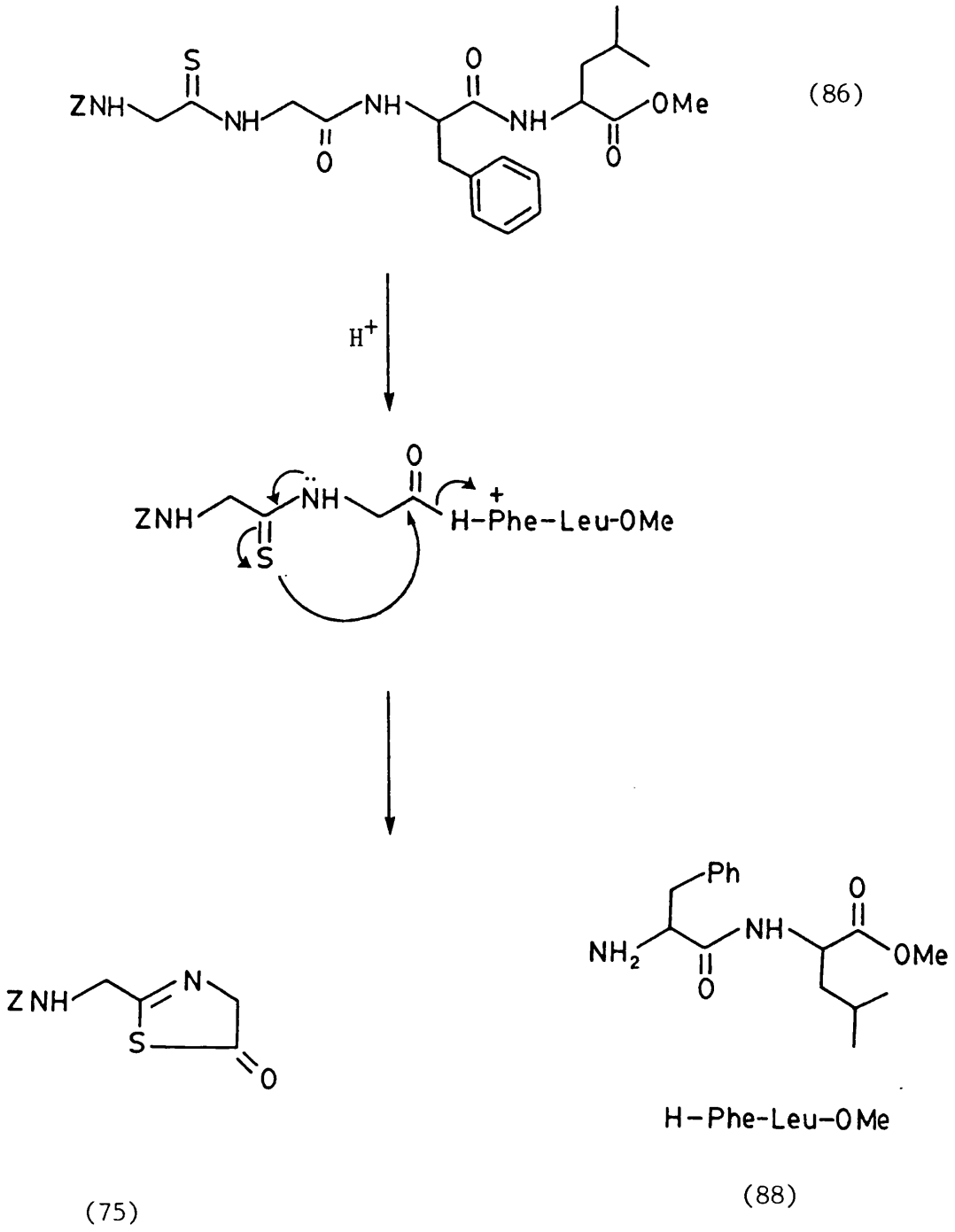
(90)



An explanation for the lower than expected yield was supplied by the isolation of the dipeptide (88) (H-Phe-Leu-OMe) from the reaction mixture (Scheme 20). This obviously results from cleavage of the Gly-Phe bond and may have been as a result of protonation followed by intramolecular cyclization of the thiono group. The 2-thiazol-5-one structure (75) was not, however, proven (Scheme 20).

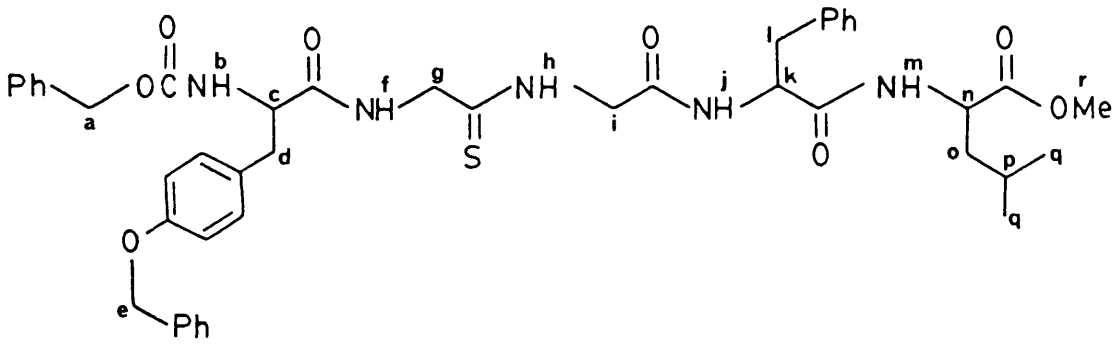
The putative tetrapeptide (90) was next coupled to Z-Tyr(Bzl)-OH (89) using the DPPCl/NMM method.[83] This gave the protected monothionated enkephalin analogue, Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87).

Scheme 20



The ^{1}H n.m.r. (400 MHz) signals for (87) are shown in Figure 5. All the assignments are as expected for a peptide of this nature except for the thioamide NH proton which is at a relatively low field value (9·7 ppm).

Figure 5: ^{1}H chemical shifts of (87)



a	4·95, 4·91	j	8·35 or 8·38
b	7·55	k	4·21 or 4·59
c	4·27	l	3·04, 2·75
d	3·04, 2·68	m	8·35 or 8·38
e	5·05	n	4·21 or 4·59
f	8·54	o	1·44-1·46
g	4·1-4·2	p	1·44-1·46
h	9·67	q	0·91, 0·94
i	4·0-4·2	r	3·60

In addition the I.R. spectrum showed three carbonyl stretching modes at 1740 (ester), 1700 (carbamate) and 1655 cm^{-1} (amide) and the U.V. spectrum a maximum absorbance at 267 nm, consistent with previously described thioamide containing peptides.[54,75]

Although this route gave the protected target compound, we were dissatisfied with several aspects of the approach. Clearly the deprotection of the tetrapeptide (86) may be an example of a problem

for the synthesis of endothiopeptides in general where a benzyloxy-carbonyl protective group and HBr/AcOH are used in conjunction. Although the key coupling reaction gives only a moderate yield, this was felt to be satisfactory enough for the purpose.

As an alternative, a second approach using a [3+2] fragment coupling was investigated.

2.4.B. [3+2] fragment synthesis of (87)

Deprotection of the starting dipeptide (55c) using the familiar HBr/AcOH conditions[78] gave the hydrobromide salt (91) (Scheme 21). The latter was identified by its ^{1}H n.m.r. and I.R. spectra and its structure confirmed by coupling with Z-Tyr(Bzl)-OH (89) using DPPCl/NMM to give the novel tripeptide Z-Tyr(Bzl)-Glyt-Gly-OPh (92) (in overall 88% yield from (55c)) (Scheme 21).

This compound was poorly soluble in most organic solvents which made large scale purification rather time-consuming. However, the clean coupling reaction made chromatography unnecessary on most occasions. In common with other tripeptides with a single thioamide link ((63), (71) and (84)) Z-Tyr(Bzl)-Glyt-Gly-OPh (92) has three carbonyl signals in its ^{13}C n.m.r. spectrum (157-172 ppm) and the thiocarbonyl carbon at 201 ppm. The ^{1}H n.m.r. spectrum again showed the thioamide NH proton at a relatively low field value (9·7 ppm).

Having obtained the left-hand segment of the required target in this convergent approach, the next coupling step with (88) was carried out as described previously (refluxing 2-propanol, TEA) but gave a poor yield (29%) of the desired protected pentapeptide (87). Yields were consistently only 25-35% in this reaction, although a higher yield (50%) was obtained on one occasion. The pentapeptide

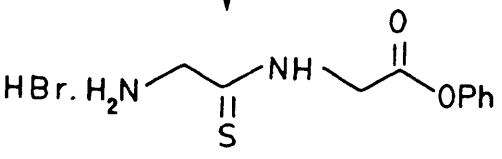
Scheme 21 : [3+2] route to (87)

(21c) Z-Gly-Gly-OPh

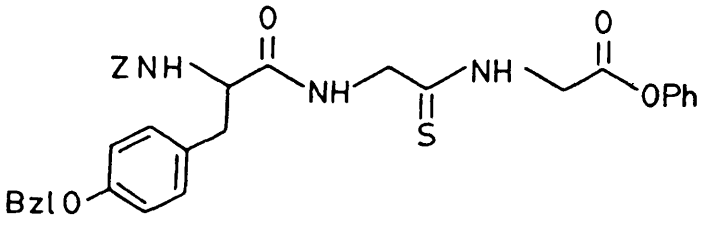
a

(55c) Z-Glyt-Gly-OPh

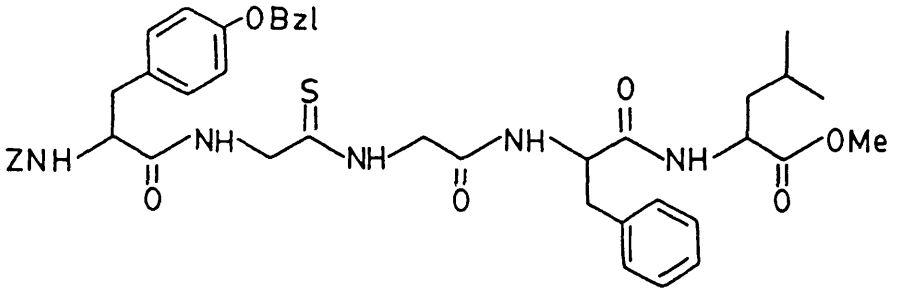
b

(91) 

c

(92) 

d

(87) 

a, (25), 80-90°C, 1 h; b, HBr/AcOH, RT, 0·5 h; c, (89), (70), NMM, -20°C, 0·5 h then (91), NMM, RT, 16 h; d, (88), TEA, 2-propanol

(87) was identical to that obtained by the [4+1] route (Section 2.4.A.).

This route circumvents the problems associated with the deprotection of (86) but gives a low yield in the last step. However, the overall yield from Z-Gly-Gly-OPh (21c) is 18% for the [3+2] route (compared to 8% for the [4+1] approach) and is preferred since only the last step is a poor one.

The phenyl ester peptides ((55c) and (92)) are only moderately reactive[90] and for this reason a slight excess of TEA and higher temperatures were used to achieve shorter reaction times. Where the C-terminal amino acid is glycine (as here) this clearly is unimportant. However, an excess of tertiary base in peptide coupling reactions is generally not desired[57,91] since racemization via the intermediate 2-oxazol-5-ones (29) can occur. In the endothiopeptide case a similar problem could be envisaged (where the C-terminal amino acid is not glycine) via the speculative 2-thiazol-5-ones (30).[55] Although no work has been carried out on this potential problem it may be overcome by using the *free* amino component instead of its salt (thus avoiding any tertiary base) although the reaction times may be lengthened by this. Another way round the problem would be to increase the reactivity of the phenyl esters by the addition of an equivalent of acid[92] and hence carrying out the reaction at room temperature. This has not been tried in this work due to lack of time but no particular problems are foreseen.

2.4.C. Attempted deprotection of Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87)

With the title compound in hand we turned to its deprotection and in this regard were unsuccessful. Several reactions were tried on a small scale (50 mg) to assess the reactivity of (87) towards various common deprotection reagents.

Treatment with trifluoroacetic acid (TFA)[80] or with a combination of TFA and trifluoromethanesulphonic acid (TFMSA)[93] gave a mixture of components by TLC and there was also evidence of the dipeptide H-Phe-Leu-OMe (88) in the TFA case.

A solution of boron tribromide in DCM;[94] and also in combination with TFA;[95] has also been used to effect removal of most protective groups. However, in our case only a slow reaction was obtained using the reagent with DCM/dimethylacetamide (necessary to dissolve the pentapeptide (87)). An initially more promising result was achieved using a boron tribromide/DCM solution with the minimum amount of TFA necessary to dissolve (87). All of the starting material was consumed after warming to room temperature (from -10°C) (2·5 h) and four components were separated by preparative paper chromatography. However, the mass spectral data on the main component (5 mg) was not conclusive; the only significant ion seen was at 261 m/e which may indicate the dipeptide H-Phe-Leu-OH($-H_2O$). The minor components (<10 mg) were not pursued.

In an attempt to remove the methyl ester group of (87), alkaline hydrolysis with sodium hydroxide (1·09 equivalents) was carried out.[84] Some pentapeptide was still present after the duration of reaction (2-3 d) along with three other components. Preparative TLC of the mixture gave the main component (excluding starting material) as an oil (9 mg) but further TLC showed it to be unstable and the mass spectral data was unhelpful.

As expected, hydrogenation deprotection methods (i.e. for removal of the benzyloxycarbonyl and benzyl ether groups) on several endothiopeptides were unsuccessful probably because of catalyst poisoning by sulphur.[96] A recently developed hydrogenation method,[97] using ammonium

formate as a hydrogen donor, when applied to (87) gave only unreacted starting material.

A considerable amount of time was spent on the deprotection of (87) since the deprotected compound would be expected to be more active.[18b] In retrospect, the presence of three different protective groups on the pentapeptide (87) is probably unwise because at least two deprotection steps would be required (although boron tribromide *is* a fairly unselective reagent), The choice of protective groups for (87) was dictated largely by the availability of the peptide materials Z-Tyr(Bzl)-OH (89) and H-Phe-Leu-OMe (88) at the time. It would be synthetically more expedient to use only one, mild deprotection step to simultaneously remove all three groups. The *tert*-butoxycarbonyl and *tert*-butyl[99] protective groups may allow this. In view of the difficulties encountered with the deprotection of (87) the synthesis of a differently protected monothionated enkephalin was carried out.

2.4.D. Synthesis of BOC-Tyr(BOC)-Glyt-Gly-Phe-Leu-OBu^t (94)

The only difference between this route (Scheme 22) and that described in Section 2.4.B. are the two peptide fragments BOC-Tyr(BOC)-OH, (95),[100] and H-Phe-Leu-OBu^t, (96).[101]

Coupling of the hydrobromide salt (91) with BOC-Tyr(BOC)-OH (95)[100] using DPPCl/NMM gave the tripeptide BOC-Tyr(BOC)-Glyt-Gly-OPh (93) (62% yield) as a crystalline solid which was considerably more soluble in organic solvents than (92) used in Section 2.4.B. and consequently easier to purify. The spectral data for (93) was completely consistent with that of previous tripeptides containing a single thioamide group, with the addition of an extra carbonyl group. The unusual tert-butoxycarbonate group carbonyl carbon could be

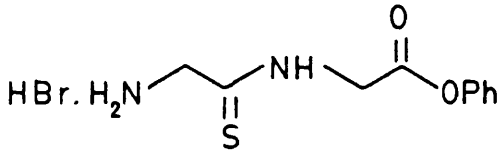
distinguished from the other carbonyl carbon signals by its high field absorbance (152 ppm) in the ^{13}C n.m.r. spectrum.

The tripeptide (93) was coupled to H-Phe-Leu-OBu^t (96)[101] following slightly different conditions (refluxing THF, TEA) to those used previously for the preparation of (84), (86) and (87) (refluxing IPA, TEA) and required a longer reaction time (27 h). After reaction some starting tripeptide (92) remained, but column chromatography gave the pentapeptide BOC-Tyr(BOC)-Glyt-Gly-Phe-Leu-OBu^t (94) (Scheme 22) in reasonable yield (68% corrected). This represents a 28% overall yield from Z-Gly-Gly-OPh (21c) and is an improvement on each of the previous routes to (87). None of the tripeptide BOC-Tyr-Glyt-Gly-OPh (97) arising from displacement of the tyrosyl phenoxy group by the dipeptide (96) or the alternative product (98), were isolated suggesting that nucleophilic attack occurs predominantly at the ester carbonyl (Scheme 23).

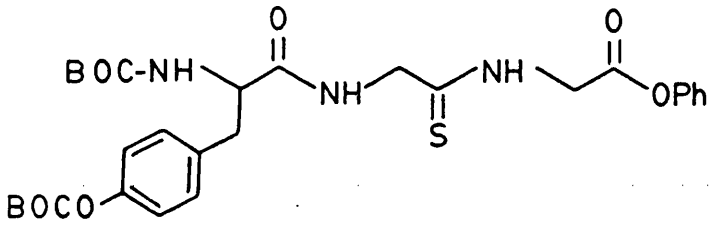
The pentapeptide (94) was completely characterized by spectral means. Its ^1H n.m.r. spectrum (400 MHz) was similar to (87) with the exception of three *tert*-butyl signals at 1·36, 1·45 and 1·54 ppm. The characteristic splitting of the *para*-substituted tyrosine aromatic ring[102] was seen at 7·10 and 7·20 ppm which was at a slightly lower field than in (87) due to the electron withdrawing effect of the t-BOC carbonate group. The thioamide proton was seen at 9·15 ppm.

The ^{13}C n.m.r. spectrum of (94) showed the thiocarbonyl carbon at 198·5 ppm with three carbonyl signals at 170-172 ppm (amide), one at 167 ppm (ester) and two more at 156 (carbamate) and 151·8 ppm (carbonate). The *tert*-butyl methyl carbons were at 27·7-28·4 ppm with the three quaternary t-butyl carbons at 80·3-83·4 ppm. All other backbone and side chain carbon signals were consistent with a peptide

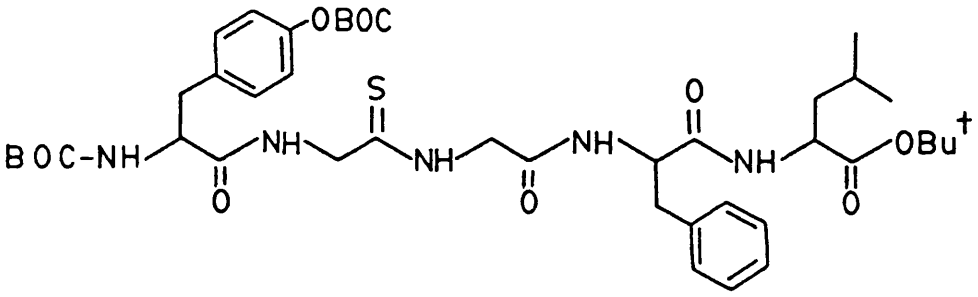
Scheme 22 : Synthesis of BOC-Tyr(BOC)-Glyt-Gly-Phe-Leu-OBu^t (94)

(91)  (Scheme 21)

a

(93) 

b

(94) 

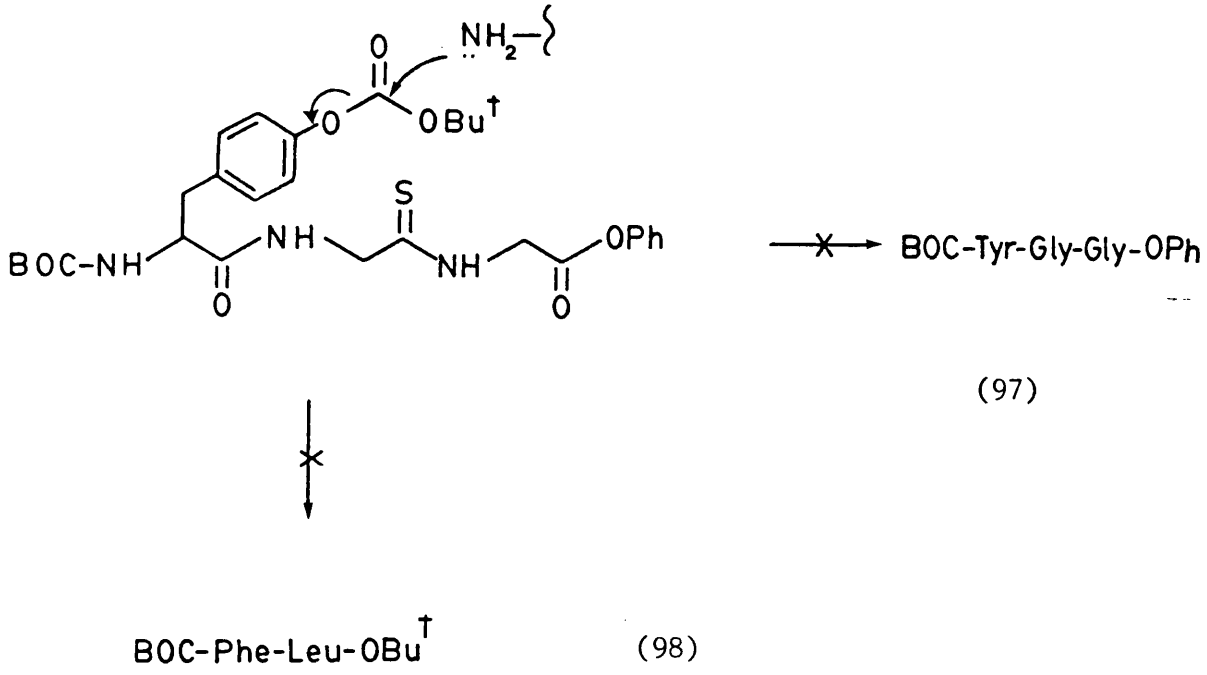
a, (95), (70), NMM, -20°C, 0·3 h then (91), RT, 16 h;

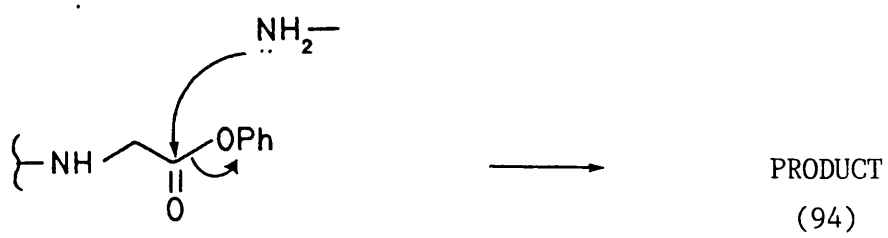
b, (96), TEA, THF, Δ, 27 h.

BOC-Tyr(BOC)-OH (95)

H-Phe-Leu-OBu^t (96)

Scheme 23



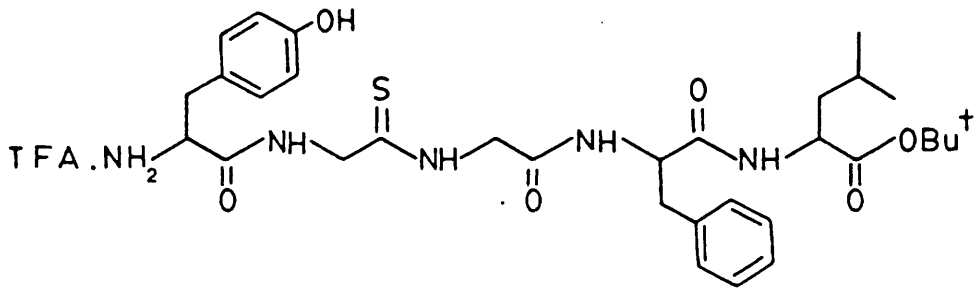


of this structure and compared closely with BOC-Tyr(BOC)-Glyt-Gly-OPh (93) and with Z-Glyt-Gly-Phe-Leu-OMe (86).

2.4.E. Deprotection of BOC-Tyr(BOC)-Glyt-Gly-Phe-Leu-OBu^t (94)

In view of the difficulties encountered with the deprotection of (87) it was gratifying to find that (94) proved somewhat more tractable. However, bearing in mind that the tert-butyl and tert-butoxycarbonyl groups normally require much milder acidic cleavage methods[98,99] (than, for instance, the benzyloxycarbonyl group) this was not completely surprising.

Indeed deprotection of (94) was achieved using trifluoroacetic acid (TFA) in dichloromethane (10%) at room temperature (1·5 h). This gave a mixture of the trifluoroacetate salt of the completely deprotected compound (83) (^{1}H n.m.r., FAB MS) (20%) and a compound with one *tert*-butyl group remaining (31%).



(99)

The latter was assigned the structure (99) based on comparison of its ^{1}H n.m.r. spectrum with that of the parent material BOC-Tyr(BOC)-Glyt-Gly-Phe-Leu-OBu^t (94) and Z-Tyr(Bzl)-Glyt-Gly-OPh (92). The latter has two doublets at 6·85 and 7·15 ppm for the para substituted aromatic tyrosyl ring whereas for a compound such as (94) with the electron withdrawing t-BOC group these are at a lower field value of 7·10 and 7·20 ppm. The mono tert-butyl compound (99) has its two doublets at 6·85 and 7·15 ppm suggesting that the carbonate group has been removed.

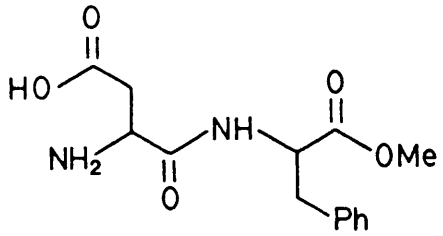
The t-BOC carbamate group is reported as being more acid labile than a *tert*-butyl ester,[103] and this would seem to infer the structure (99) for the mono tert-butyl compound.

The ^{1}H n.m.r. spectrum (400 MHz) of the trifluoroacetate salt of (83) was fully consistent with its structure and its FAB mass spectrum virtually identical in all key features to other reports.[42]

2.5. RECENT RELEVANT WORK

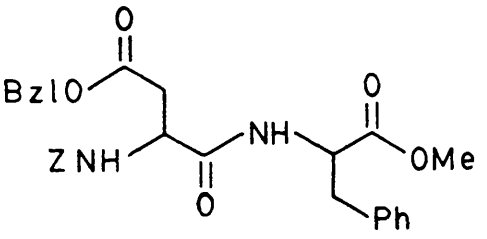
The use of Lawesson's reagent (25) as a high yielding thionating agent[49,50] and its application to the synthesis of thioamides[51] has led to renewed interest in endothiopeptides. Our own work[75] and the independent work of Lawesson's group[54] has already established that simple N-protected dipeptide esters (21) can be readily and selectively thionated at the amide link. Even more recently and concurrent with this work, reports have been published of other thionated analogues of important peptides.

The commercially available sweetener 'Aspartame' is the dipeptide ester H-Asp-Phe-OMe (100).[104] Two N-protected thionated



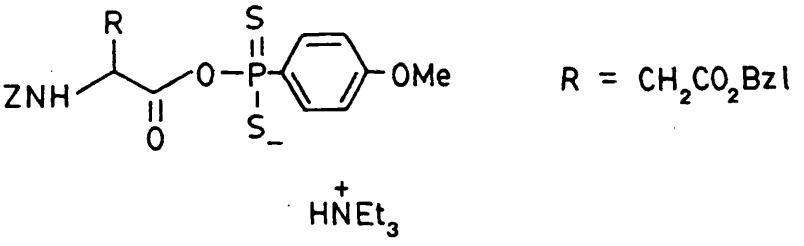
(100)

analogues were prepared,[43a] using (25), from the normal oxygen peptides. Interestingly, one of the latter (101) was prepared using (25) as a



(101)

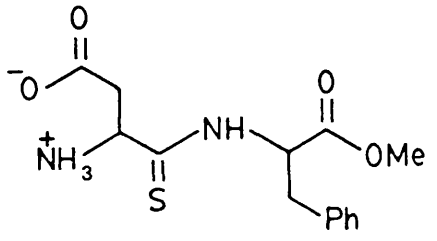
peptide coupling agent, probably via the mixed anhydride species (102).[105]



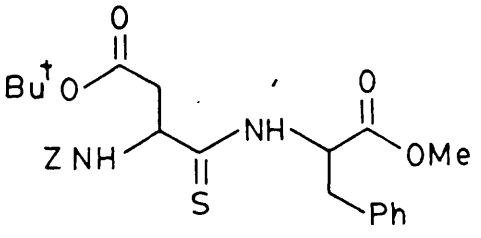
(102)

The deprotected endothioaspartame (103) was obtained via acid treatment of the derivative (104).

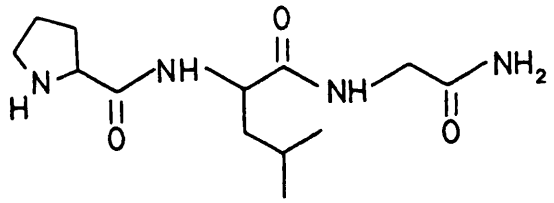
(103)



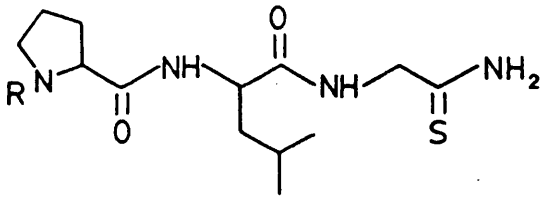
(104)



The three monothionated analogues of the tripeptide amide, H-Pro-Leu-Gly-NH_2 (105) (Melanostatin - a neurologically active peptide derivative) have also been made.[43b] Two methods for the introduction of the thioamide group were used. For H-Pro-Leu-Glyt-NH_2 (106b) Lawesson's reagent (25) was used to obtain BOC-Glyt-NH_2 (107) from its

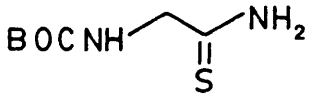


(105)



(106a) R=BOC

(106b) R=H

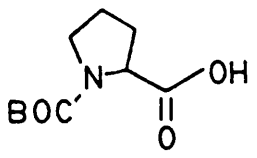


(107)

oxygen analogue. Subsequent deprotection (HCl/acetone) and coupling with BOC-Pro-Leu-OH (108) (using (25) as a coupling agent) gave the tripeptide (106a). This was then also deprotected, to give (106b).

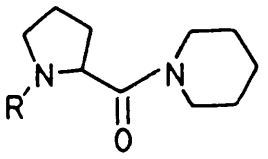
A similar direct thionation procedure, followed by deprotection, coupling and aminolysis gave the second endothiomelanostatin analogue (108).

Scheme 24 : Preparation of HCl.H-Prot-Leu-Gly-NH_2 (115)[43b]



(109)

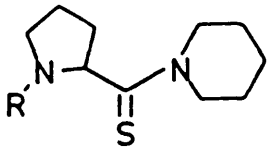
a



(110)

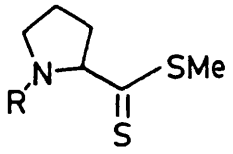
R = BOC

b



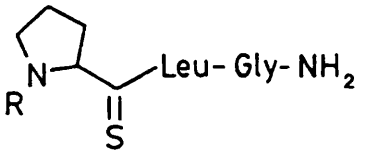
(111)

c , d



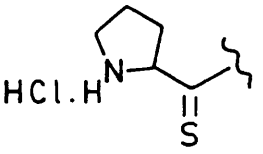
(112)

e



(113)

f

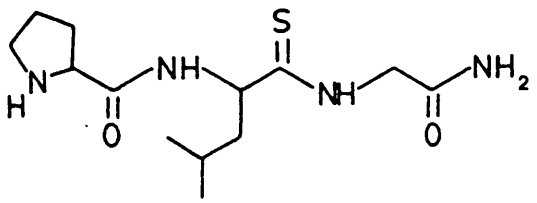


(114)

H-Leu-Gly-NH_2 (115)

a, (25), TEA, RT, 0·1 h then Pip-H, -15°C, 0·25 h, RT, 16 h;

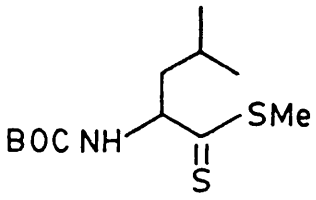
b, (25), PhH, Δ; c, MeI, THF, RT; d, H_2S, MeOH, 0·5 h; e, (115), TEA, RT, 16 h; f, HCl, dioxane, RT, 0·5 h.



(108)

For the H-Prot-Leu- derivative (115) a completely new procedure was used to introduce the thioamide groups indirectly (Scheme 24). The dithioester (112) was used as a thioacylating agent. Dithioesters of this type have been used only briefly for the synthesis of endo-thiopeptides previously.[40] In this case the dithioester (112) was obtained in four steps from the amino acid derivative, BOC-Pro-OH (109). The piperidide (110) was prepared, using (25) to form the intermediate mixed anhydride species (102), ($R \neq CH_2CO_2Bzl$) which reacted exothermally with piperidine. Thionation of (110) proceeded exclusively at the amide carbonyl as expected giving the thiopiperidide (111). In a 'one-pot' procedure, the latter (111) was S-methylated with excess methyl iodide and then thiolysed with hydrogen sulphide giving the dithiomethyl ester (112) in reasonably good overall yield from (109) (21%). On reaction with the dipeptide ester (115) the tripeptide BOC-Prot-Leu-Gly-NH_2 (113) was obtained (56%) and a straightforward deprotection gave the monothio analogue (114).

Originally the latter approach was also going to be used to obtain the Leut-Gly derivative (108) but the appropriate dithiomethyl ester (116) was almost completely unreactive even under more forcing conditions.[43b] The large difference in reactivity may be due to the greater steric bulk of the leucyl side chain compared to the rigidly

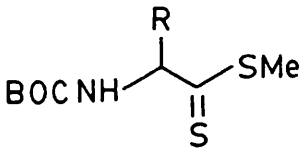


(116)

held prolyl molecule. Although the leucyl dithiomethyl ester did not give the desired results, this method has obvious application to other amino acids.

In fact, during the last few months Lawesson's group has extended this approach to endothioenkephalins.[42] All four suitably protected monothioenkephalin derivatives have been made and three of these (Tyrt-Gly, Glyt-Gly and Phet-Leu) have been deprotected to the free endothioamino acids. Only the Glyt-Phe compound has not been obtained as the deprotected material. This recent work and that described in this Thesis are complementary in nature since the approaches used are different.

Lawesson appears to favour an indirect method for introduction of the thioamide group, using dithioesters of BOC-amino acids (117)



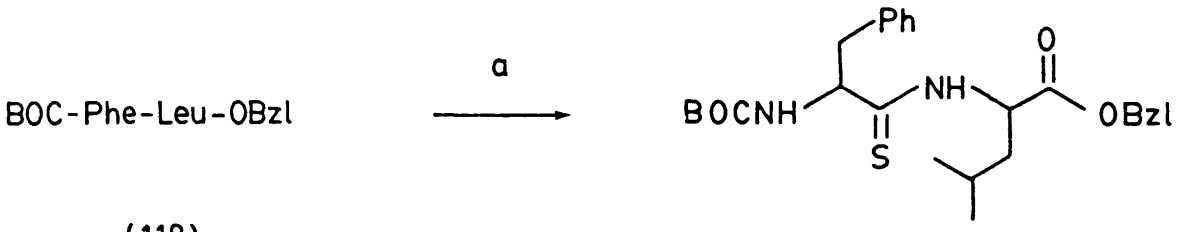
(117)

as thioacylating agents, although for the Phet-Leu compound (121) a

direct method was used. Thus the N-protected dipeptide ester (118) was thionated with Lawesson's reagent (25) giving the endothiopeptide (119) (Scheme 25). The latter was deprotected and coupled to the tripeptide (122). Final deprotection using hydrogen fluoride and anisole (as a cation scavenger)[106] gave tne totally deprotected monothioenkephalin, (121). The latter method was one of the few not tried in the deprotection of Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87) although slightly different protective groups were used by Lawesson. This route is a direct thionation approach (used for the Glyt-Gly compound and described in this Thesis) and gives a good overall yield (from commercially available BOC-Phe-OH) to the protected pentapeptide (68%). It is slightly surprising that it was not used for the other three endothioenkephalin analogues.

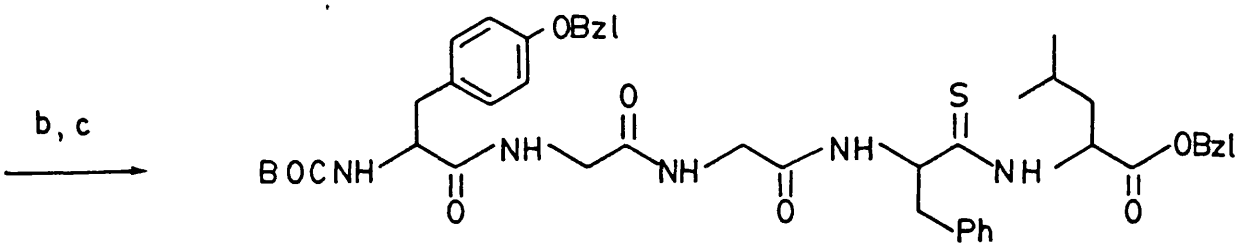
Instead, for the latter, thioacylation approaches were investigated by Lawesson and co-workers. The thioester method, first used by Ried *et al.*[34,35,38] (see Introduction), was applied to the synthesis of two endothiopeptides Z-Glyt-Gly-Phe-Leu-OBzl (123) and Z-Glyt-Phe-Leu-OBzl (124). However, the N-protected thioester used, Z-Glyt-OEt (125) gave only complex mixtures when reacted with the required free amino peptides, although it could be used successfully for the preparation of *smaller* N-protected dipeptide esters like (55) but in only moderate yields (35-38%). In any case this route is probably unsatisfactory for chiral amino acids because of the nature of the synthesis of the starting aminonitrile.[36]

Scheme 25

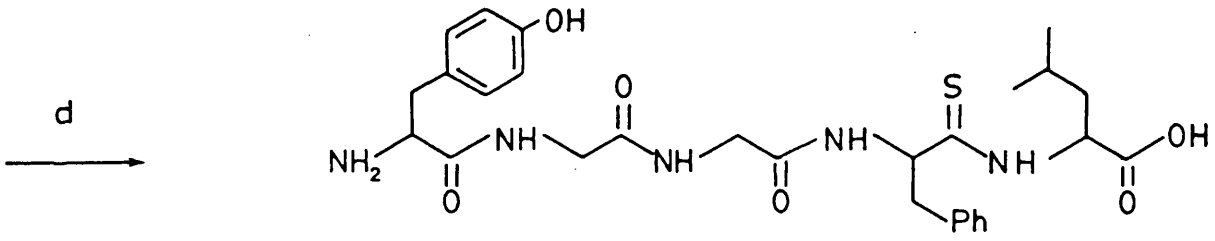


(118)

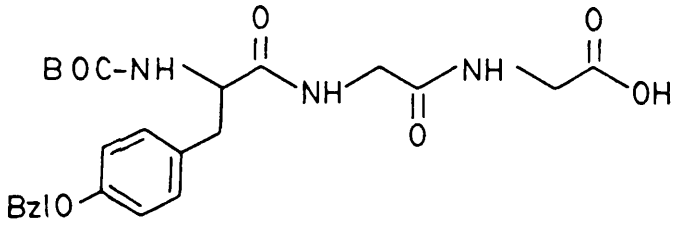
(119)



(120)



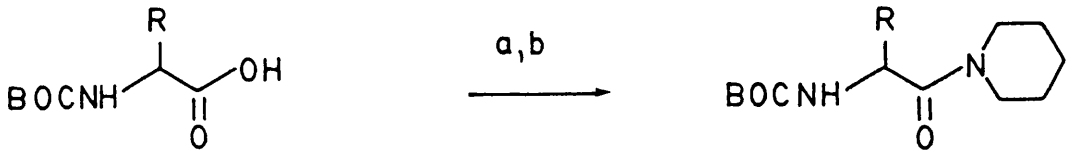
(121)



(122)

a, (25), PhH, Δ; b, HCl, dioxane, RT, 0·5 h; (122), DCC, -10°C, 1 h, RT, 10 h; d, HF, $PhOCH_3$, 0°C, 0·5 h.

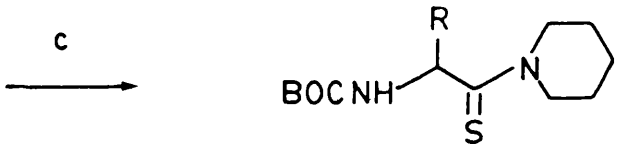
Scheme 26



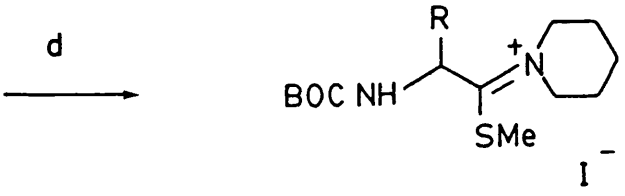
(126) (127)

a : R = H

b : R = CH_2-C$_6$H$_4$-OBzl



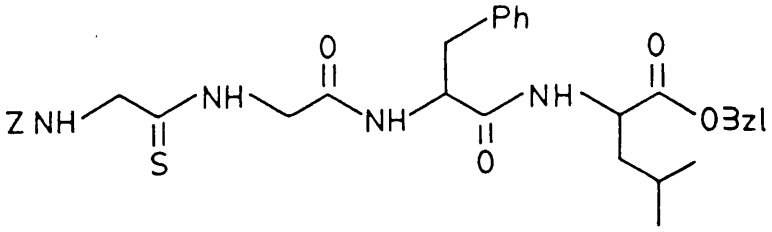
(128)



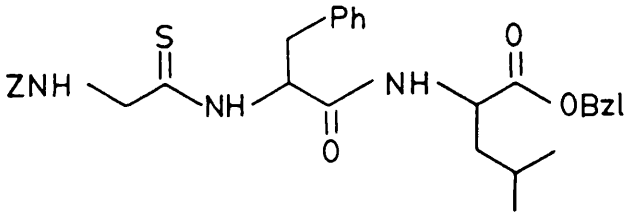
(129)

(130)

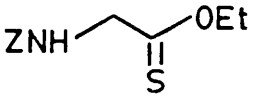
a, (15), TEA, RT, 0·1 h; b, Pip-H, -15°C, 0·5 h, RT, 4 h; c, (25), PhH, Δ; d, MeI, THF, RT, 12 h, e, H_2S, EtOH, 0°C, 0·7 h.



(123)



(124)



(125)

Next, the use of N-protected amino acid dithioesters was further examined (previously described for Melanostatin[43b]) and found suitable for the enkephalin case. The use of N-benzyloxycarbonyl amino acid dithioesters, however, gave problems in subsequent deprotection steps (this is in accord with our findings on the removal of N-benzyloxycarbonyl groups) and therefore N-*tert*-butoxycarbonyl amino acid dithiomethyl esters (130) were used (Scheme 26). The most convenient preparation method was the four step route, outlined previously for

the Melanostatin endothio analogues, shown in Scheme 26.

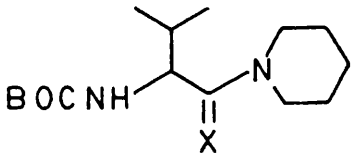
All the steps in the sequence proceed in good yield (56-91%) and the intermediate methiodides (129) were isolated in this case. The dithiomethyl esters (130) were reactive thioacylating agents and on coupling with the required free amino peptide esters by either stepwise or fragment procedures gave the suitably protected (BOC amino, benzyl ether and benzyl ester) monothioenkephalins (Tyrt-Gly, Glyt-Gly and Glyt-Phe). The final step in all cases used hydrogen fluoride/anisole (106) as the deprotection agent. However, for the Glyt-Phe derivative (Z-Tyr(Bzl)-Gly-Glyt-Phe-Leu-OBzl) the deprotected compound could not be isolated and the method failed in this instance.

The overall yields, from commercially available starting materials, for the fully protected pentapeptides in the Tyrt-Gly and Glyt-Phe cases are quite respectable (30% and 38%) but the latter compound has not been deprotected. For the Glyt-Gly example the overall yield is not significantly different (29%) to the work described herein for BOC-Tyr(BOC)-Glyt-Gly-Phe-Leu-OBu^{t} (94) (26%). The best yield is for the Phet-Leu example (120) where the direct nature of the route is reflected in the higher value (68%).

The fact that Lawesson's group used the indirect method for thioamide incorporation and thus avoided all attempts at carboxyl terminus deprotection until the final stage may reflect the difficulties encountered in this regard (also described in Section 2.2. and by Lajoie *et al.*[55]). The Phenyl ester method circumvents this problem.

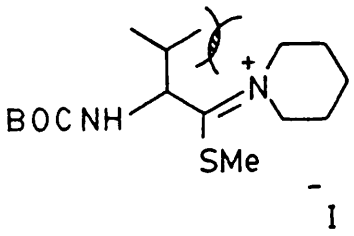
In connection with the work described in the following section (2.6.) we have since found that the indirect method of introduction

of the thioamide group used by Lawesson[43] is unsatisfactory for N-protected amino acids with 'bulky' side chains (e.g. valine). Thionation of the piperidide of BOC-Valine, (132a) with (25) proceeded only slowly and gave a poor yield of product (132b) (30%). Furthermore, the attempted methylation of (132b) following Lawesson's work does not



(132) a : X = O
b : X = S

proceed even when more vigorous refluxing conditions are applied (although stronger methylating agents have not been tried). It may be that the steric bulk of the valyl side chain prevents the C-N bond becoming more planar in the formation of the methioidide (133).



(133)

The use of a "modified Lawesson's reagent" (27a) has been reported recently[55] and, in fact, a fully protected Glyt-Gly enkephalin (131b) has been prepared in one step from the pentapeptide (131a)

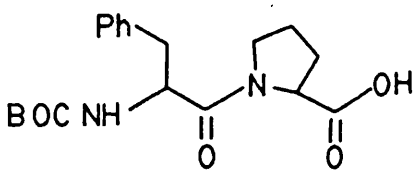
rapidly (3·5 h) and in good yield (75%).

(131) BOC-Tyr(BOC)-Gly-Gly-Phe-Leu-OMe (X = O or S)

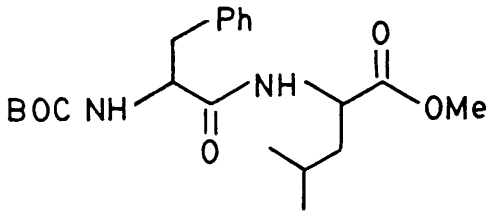
a X=O

b X=S

However, other methods to introduce the thioamide group at more sterically 'crowded' amide links will probably be required. Indeed the phosphetane disulphide (27a) (Belleau's reagent") gave lower yields with more hindered amino acid peptides (for example (134) and (135)), and also required higher temperatures (40, 50°C) and longer



(134)

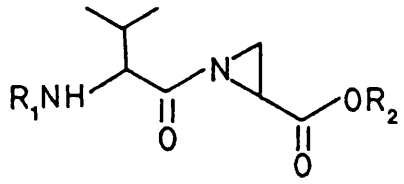


(135)

reaction times (24, 48 h), than for glycine containing peptide derivatives (0-23°C, 0·1-3·5 h).

2.6. APPROACHES TOWARDS THE SYNTHESIS OF ENDOTHIOAZIRIDINE PEPTIDES

The progress of the work aimed at the synthesis of the cyclic peptide Ascidiacylamide[63] is discussed below. The retro-synthetic scheme (Scheme 9) outlined in the Introduction focused attention on the endothioaziridine peptide unit (37) and our initial efforts were directed at this target.

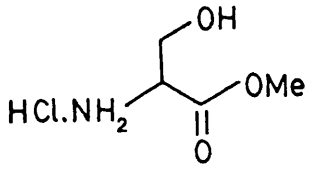


(39) X=O
(37) X=S

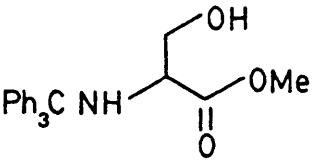
It seemed likely that the direct thionation of a suitably protected derivative of type (39) (probably prepared by peptide coupling of a valine derivative with a suitable aziridine) would give the desired endothioaziridine structure (37), although the stability of these systems was not known.

The 2-aziridine carboxylic *acid* derivative (139) was prepared in a three step sequence from serine methyl ester hydrochloride salt (136) (Scheme 27) following the route used by Japanese workers and described in detail[67,68] for the 3-methyl-2-aziridine carboxylic acid derivative (140). This sequence applied to serine methyl ester (136) gave N-triphenylmethyl-2-aziridine carboxylic acid methyl ester (139) in a high degree of purity and in excellent overall yield. Although the preparation of aziridine (139) *is* described in the literature[67,68] its spectroscopic details are absent. *Peptides* containing the 'Azy' unit have however been described.[107] The ^{1}H n.m.r. spectral data of

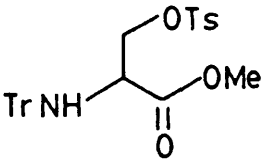
Scheme 27



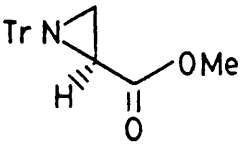
(136)



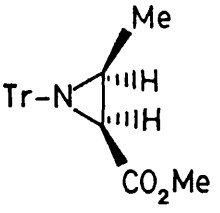
(137)



(138) Tr = Ph_3C



(139)

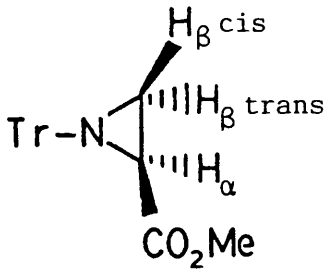


(140)

a, Ph_3 CCl, TEA, 0°C, 2 h, RT, 18 h; b, Ts Cl, py, -10°C, 2·5 d;

c, TEA, THF, Δ, 2·5 d.

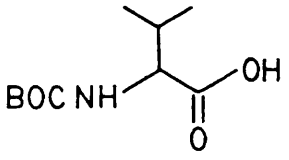
(139) showed the aziridinyl protons clearly as doublets of doublets at 1·4 (βH, trans), 1·9 (αH) and 2·3 ppm (βH, cis). The coupling constants were used to aid the assignments. The geminal coupling between the β



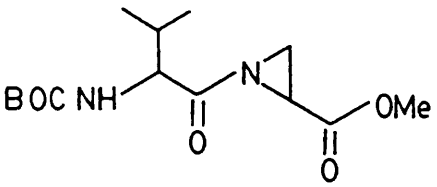
(139)

protons was small (1·5 Hz)[108] owing to the effect of the ring environment. The coupling between the βH (cis) and αH was less (2·5 Hz) than that between the other βH (trans) and the αH (6 Hz).

The aziridine is stable in its protected form and could be stored indefinitely at room temperature without adverse effects. No untoward difficulties were encountered with its handling provided precautions were taken to avoid exposure to agents likely to open the ring (for example, HCl).

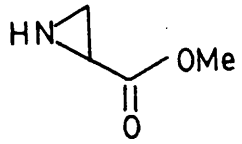


(141)



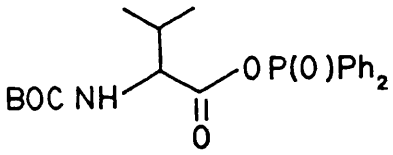
(142)

Next, the aziridine (139) was deprotected and coupled to BOC-Val-OH (141) to furnish the novel dipeptide BOC-Val-Azy-OMe (142). This was accomplished most conveniently, without isolation of the deprotected aziridine (143), using a solution of TFA (33%) in



(143)

$MeOH/CHCl_3$ at room temperature and subsequent reaction with the mixed anhydride (144). The latter is easily formed from BOC-Val-OH (141)



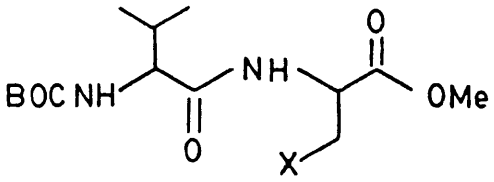
(144)

and DPPCl (70) at low temperatures (-20°C). This two step sequence gave a moderate overall yield (39%) of the dipeptide (142). The latter clearly shows the aziridine protons still present in the ^1H n.m.r. spectrum at 2·7 (β protons) and at 3·25 ppm (αH) although they have been shifted downfield compared to (139) by the electron withdrawing 'acyl' group. The ^1H n.m.r. spectrum also shows the valyl methine protons at 2·2 ($CH\ Me_2$) and 4·2 ppm (NH CH) with the latter coupled (J = 9 Hz) to the carbamate NH proton.

The ^{13}C n.m.r. spectrum of this dipeptide has three carbonyl

carbon signals at 155·7, 168·6 and 182·8 ppm consistent with the carbamate, ester and amide groups of an aziridine dipeptide. The double bond character of the C-N bond is reduced, compared to 'normal' peptides, as evidenced by the downfield shift of the amide carbon (which usually resonates at ~170 ppm in more conventional peptides). Other signals observed are as expected for a valyl amino acid residue with the aziridine ring carbons at 30·6 (CH_2 N) and 34·4 ppm (CH N).

The lower than expected yield in this reaction can be partly explained by the isolation of another dipeptide product (9%) from the reaction. This was identified as a valyl-alanine derivative (145) arising from ring opening of the aziridine by a nucleophilic species (X). The identity of the nucleophile was thought to be a chloride ion

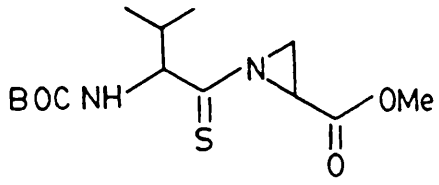


(145)

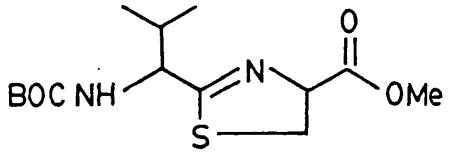
which is only present in the reaction mixture as N-methylmorpholine hydrochloride. This may, however, be sufficient to cause ring opening. Evidence for N-tert-butoxycarbonyl-β-chloroalanine was provided by ^{1}H n.m.r. and ^{13}C n.m.r. spectra which were similar to the spectra for BOC-Val-Ser-OMe.[109] The mass spectrum of (145) showed several ion pairs of two mass units difference in the ratio of 3:1 (isotopic ratio of ^{35}C:^{37}C) although the molecular ion did not show this feature and this cannot be explained.

With the desired aziridine peptide (142) to hand we investigated

its thionation with a view to forming the endothioaziridine (146). It was suspected, from a literature comparison with thioacyl aziridines,[64,65] that the postulated product (146) would rearrange under the reaction conditions to the 2-thiazoline (147) .



(146)



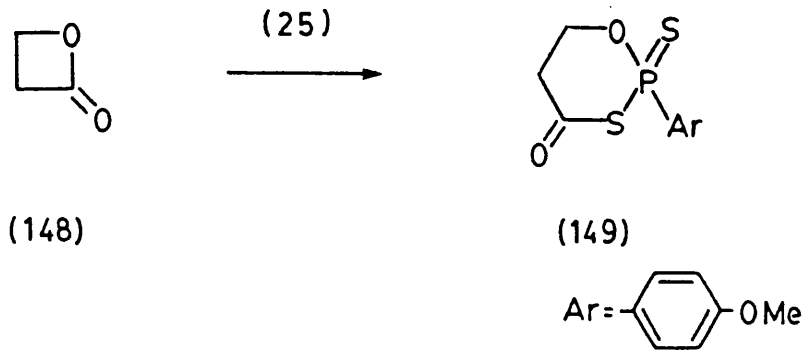
(147)

However, treatment of the aziridine peptide (142) with Lawesson's reagent (25) at elevated temperatures (80°C) was unsuccessful. Although one reaction gave, by TLC analysis, an unstable product that was different from the starting dipeptide (142), this could not be repeated. This product had a ^{1}H n.m.r. spectrum that was consistent with the endothioaziridine dipeptide (146) and, as expected, this was similar to starting material (142).

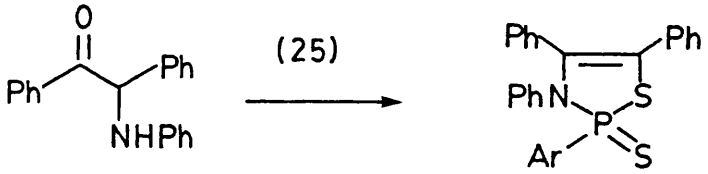
Milder thionation conditions were employed using the alternative phosphetane disulphide (27a).[55] The attempted thionation of BOC-Val-Azy-OMe (142) at room temperature with "Belleau's reagent" (27a) did not yield the suspected endothioaziridine (146), obtained above, but gave, after column chromatography, a more polar product. Although this was originally thought to be a single component, closer examination revealed it to be a mixture which could not be separated. The

^{1}H n.m.r. spectrum showed the presence of both aromatic protons (7-8 ppm) and those attributable to the BOC-Val- segment of the aziridine dipeptide (142). The aziridine ring protons were not seen. In an attempt at further resolution a high field (400 MHz) spectrum was obtained but this showed the sample to be even more complex than at first thought and was not helpful.

Lawesson's reagent (25) is known to form insertion products with small membered rings. For example, β-lactones (148) give unusual heterocycles (149).[110] More relevantly, perhaps, the α-amino ketone (150) gave the 2,3-dihydro-1,3,2-thiaza-phosphole (151).[111] The corresponding 1,3,2-thiazaphospholidine-2-oxides (152)[112] and 2-sulphide-4-carboxylic acids (153)[113] have been prepared by other routes, the latter from an amino acid derivative and a suitable phosphorus reagent.

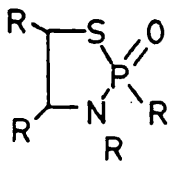


(148) (149)

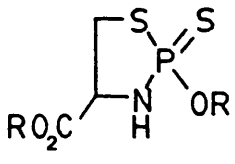


(150) (151)

The structure of the product from the reaction of BOC-Val-Azy-OMe (142) with Belleau's reagent (27a) is (very) tentatively proposed as the 1,3,2-thiazaphospholidine-2-sulphide-4-carboxylic acid derivatve

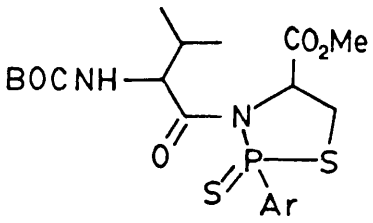


(152)



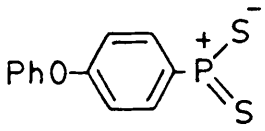
(153)

(154). The latter might be formed by attack of the phosphetane sulphide species (155)[111] on the aziridine ring of the dipeptide (142)



(154)

and subsequent ring opening. In support of this structure the ^1H n.m.r.



(155)

spectrum contains the t-butyl, valyl, methyl ester and aromatic protons in the correct integral ratios. The mixture of products may be explained by a mixture of diastereoisomers at the chiral centres of (154) (including phosphorus) and this may also go some way to explaining the complexity of the ^1H n.m.r. spectrum. However, the product may be unstable, which would complicate the correct assign-

ment of chemical shift values. Thiazaphospholidine oxides (152) are reported to be susceptible to hydrolysis.[112]

The mass spectrum (CI) of the product also supports the structure (154). The highest significant ion was observed at m/e 464 which is 100 mass units lower than the molecular ion of structure (154). However, loss of the *tert*-butoxycarbonyl group from (154) would explain this result. Another fragment ion is seen at m/e 448 which may correspond to a further loss of NH_2- from the 464 fragment. The base peak at m/e 105 and other significant ions (331, 278) are, however, unexplained.

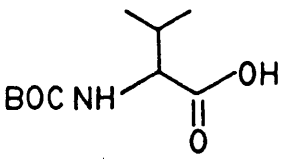
In view of these results, we turned to the use of phosphorus pentasulphide (P_2S_5) as a thionation agent. A recent application[44] used ultrasound to improve the homogeneity of the reaction medium and this excluded the need for high temperatures. Under these conditions[44] the starting dipeptide was consumed within a short time (<3·5 h). However, after an aqueous work-up, and column chromatography to obtain the non-polar materials, a poor yield (~35%) of a colourless oil was obtained that did not chromatograph cleanly. This suggested a mixture and/or unstable product(s).

The ^1H n.m.r. (60 MHz) of the oil showed the t-butyl, valyl and methyl ester protons (in the correct integral ratio) but the rest of the spectrum was not fully resolved. A mass spectrum (CI) of the product(s) showed an ion at m/e 317 which is the molecular mass (+1) of the 2-thiazoline (147). Although other fragment ions (261, 217), consistent with the thiazoline (147) were seen, the base peak at m/e 279 could not be explained by this product. The base peak may have arisen from a higher m/e value fragment or molecular ion (m/e 335) by the loss of 2-butene (C_4H_8) and this seems to suggest a mixture of

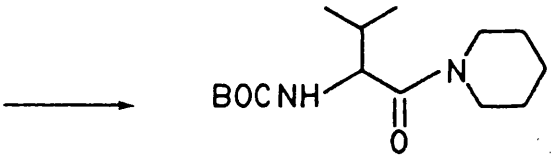
products.

The use of an indirect route for the incorporation of the thioamide functionality was next explored since a clean product could not be obtained from the P_2S_5 method. Lawesson's group[42,43] have used dithioesters as thioacylating agents and this was investigated. The required valyl dithioester derivative (156) should be available in moderately high yields using similar methodology (Scheme 28).

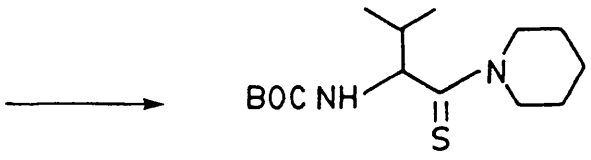
Scheme 28



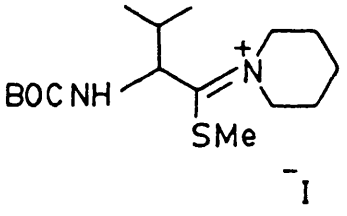
(141)



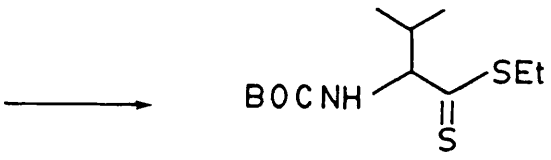
(132a)



(132b)



(133)



(156)

The novel piperidide (132a) was prepared in a high yielding step (95%) from BOC-Val-OH (141) using DPPCl (70) and NMM to form the mixed anhydride species (144) *in situ* and subsequent reaction with piperidine. This peptide coupling agent gives consistently clean, efficient reactions when used with nitrogen nucleophiles. The piperidide (132a) had fully consistent n.m.r., IR and mass spectra.

Thionation of the latter product (132a) with Lawesson's reagent (25) was quite sluggish, compared to other thionation reactions, and the thiopiperidide (132b) was only formed in poor yield (23%). Some of the starting piperidide (132a) was also recovered (17%). The product was characterized by its ^{1}H n.m.r. and ^{13}C n.m.r. spectra. The latter showed the appearance of a thiocarbonyl carbon signal (202·7 ppm) when compared to the piperidide (132a). The amide carbon of the latter (170·2 ppm) was not present in the sulphur analogue (132b).

The attempted methylation of the thiopiperidide using methyl iodide (excess) in THF following an identical method to that of Lawesson *et al.*[42,43] gave unreacted starting material even under more vigorous conditions (refluxing THF). Since it was suspected that the methiodide (133) would not be chromatographically stable, the reaction mixture was evaporated and the ^{1}H n.m.r. spectrum of the residual oil obtained. This, however, was identical with that of the starting thiopiperidide (132b).

The reason for the lack of reactivity of the thiopiperidide (132b) towards methylation is not clear. The steric bulk of the valyl side chain may create an undesirably crowded environment in the methiodide (133). The enforced planarity of the C=N double bond may be sufficiently unfavourable to prevent product formation. However,

the planarity of the C-N bond would also be present in the thioamide (132b) to a considerable degree and thus this reason does not seem completely justified.

Several approaches for the synthesis of the endothioaziridine (146) from a suitable peptide derivative using methodology described earlier in this work have been explored. Although it is suspected that the endothioaziridine (146) is not particularly stable, other products have not been proved. The aziridine ring of the dipeptide (142) does not seem sufficiently stable to allow thionation with phosphetane disulphides ((25) and (27a)) and this is clearly a limitation to their use. We have shown that the most synthetically useful indirect method[42,43] for incorporation of the thioamide group appears to be also limited, by sterically encumbered amino acids. The phosphorus pentasulphide method is the most promising but lack of time has precluded a further investigation of the products obtained.

2.7. CONCLUSION

This work has described suitable procedures for incorporation of the thioamide functionality into small biologically important peptides (the enkephalins) which will allow other thioamide analogues of peptide molecules to be synthesised and investigated. During the course of the work various peptide coupling and deprotection methods have been investigated for their suitability in these new systems.

The choice of protective groups for these analogues is important when larger (tri, tetra and penta) peptides are required. Two protected enkephalins with the Gly-Gly amide bond replaced by a thioamide have been prepared by different routes. The deprotection of one of the endothioenkephalins has been achieved giving the free amino

endothiopeptide acid (83).

The use of endothiopeptides as synthetic intermediates for heterocyclic peptide synthesis has been investigated. This has been successful as a preliminary exploration and points the way to other efficient applications.

EXPERIMENTAL

SOLVENTS AND REAGENTS

Solvents were distilled and dried, prior to use, by standard methods,[114] when required. Reactions involving moisture sensitive materials were carried out under an inert atmosphere of dry nitrogen. All evaporations were carried out *in vacuo* below 60°C.

Most amino acid and peptide derivatives used as starting materials were commercially available. Other derivatives were prepared by literature methods as cited. The latter were homogeneous by TLC and ^{1}H n.m.r. The author thanks ICI Pharmaceuticals Division (Macclesfield) for gifts of other selected starting materials.

CHROMATOGRAPHY

TLC was carried out using commercially available pre-coated silica gel 60 F_{254}, or 'reverse phase' silica, plates. Visualizations in both cases were achieved by ultra-violet fluorescence at 254 nm and/or the following spray reagents[115]:

Ninhydrin (free NH_2)

Bromocresol green (CO_2H)

Phosphomolybdic acid (general)

Anisaldehyde (general)

Iodine vapour (general).

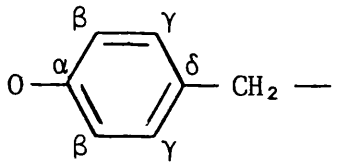
Medium pressure column chromatography was carried out using silica gel (Merck 7747) or modified 'reverse phase' silica.[116]

SPECTROSCOPY

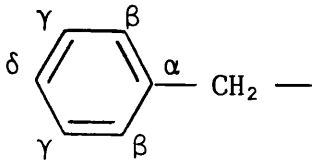
Nuclear magnetic resonance (n.m.r.) spectra were recorded at 100 MHz (^{1}H) or 22·5 MHz (^{13}C) unless stated otherwise. All spectra

were run in deuteriochloroform ($CDCl_3$) or deuterium oxide (D_2O) with tetramethylsilane (TMS) or *p*-dioxane as internal standards unless specified otherwise. Chemical shifts (δ) are expressed downfield from TMS in all cases with multiplicities denoted by s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), dd (doublet of doublets) or m (multiplet). Assignments are expressed as the atom (or group) concerned followed by, either, its adjacent atom (or group) or, the amino acid residue (of the peptide) in which it occurs. For the ^{13}C n.m.r., spectral multiplicities are those observed in the OFR (off field resonance) spectra.† Aromatic carbons were assigned according to the following labelling of the ring atoms:

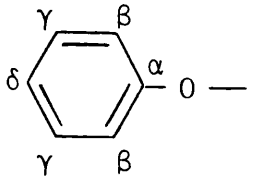
Tyrosyl



Phenylalanyl



Phenyl ester



† Where this information was obtained by an INEPT (insensitive nuclei enhancement by polarization transfer) spectrum[117] the data is presented as if an OFR spectrum had been run.

Infra-red (IR) spectra were recorded as Nujol mulls with the absorption frequencies (ν) expressed in cm^{-1}.

Ultra-violet (UV) spectra were recorded as ethanolic solutions unless stated otherwise. Maximum absorptions (λ_{max}) are expressed in nm with the molar absorptivity (ε) as 10^{-2} m^2 mol^{-1}.

Mass spectra (MS) were recorded using electron impact (EI) at 70 eV unless otherwise stated.

The author thanks the Science and Engineering Research Council (SERC) for use of the high field (400 MHz) n.m.r. facility at the University of Warwick.

OTHER TECHNIQUES

All melting points are uncorrected.

Elemental analyses were carried out by Butterworth Laboratories (Teddington) and the accurate mass measurements obtained by the Physical and Chemical Measurements Unit (Harwell).

INSTRUMENTATION

^1H n.m.r.	J.E.O.L. PS 100 (100 MHz)
	Varian EM360 (60 MHz)
	Perkin-Elmer R24B (60 MHz)
^{13}C n.m.r.	J.E.O.L. FX 90 Q (22·5 MHz)
I.R.	Perkin-Elmer 197 and 1310
U.V.	Perkin-Elmer 402 and Lambda 3

M.S.	VG 7070E with VG 2000 data system
mp	Electrothermal

METHODS

Thionation of N-protected dipeptide ester substrates (21)

The preparation of Z-Glyt-Gly-OPh (55c) and Z-Alat-Ala-OMe (55e) are given as examples of the thionation reaction. The experimental details for other N-protected endothiodipeptide esters are shown in Table 5. Unless otherwise stated, all thionations were carried out in dry toluene at 80-90°C (0·5-2 h) using Lawesson's reagent (25) (0·55 molar equivalent).[48] Medium pressure column chromatography of the reaction mixture after evaporation gave the products (55) and (23) as white solids or colourless gums.

N-Benzyloxycarbonyl-thioglycyl-glycine phenyl ester (55c)

A suspension of Z-Gly-Gly-OPh (21c) (2·57 g, 7·5 mmol)[75,118] and Lawesson's reagent (25) (1·6 g, 4 mmol) in dry toluene (50 ml) was heated (1 h) at 80-90°C. The resulting solution was cooled to room temperature and the solvent evaporated giving an oily semi-solid residue. The crude material was subjected to medium pressure column chromatography (5% EtOAc/DCM) and the fractions containing the desired product (R_f 0·3) combined and evaporated. This gave the title compound as a slightly off-white solid (1·90 g, 70%).

mp 92·5-94·0°C

^{1}H n.m.r. δ 4·20 (2H, d, 6 Hz, CH_2 Glyt^1), 4·55 (2H, d, 5 Hz, CH_2 Gly^2), 5·10 (2H, s, CH_2O), 5·80 (1H, br t, NH Glyt^1), 7-7·40 (10H, s, m, Ar), 8·70 (1H, br s, NH Gly^2).

^{13}C n.m.r. δ 47·0 (t, CH_2 Gly^2), 52·0 (t, CH_2 Glyt^1), 67·5 (t, CH_2O), 212·2 (d, C_β, OPh), 126·4 (C_δ, OPh), 128·0 (C_β, $\underline{Ph}CH_2$), 128·3 (C_δ, $\underline{Ph}CH_2$), 128·6 (C_γ, $\underline{Ph}CH_2$), 129·6 (C_γ, OPh), 136·1 (s, C_α, $\underline{Ph}CH_2$), 150·2 (s, C_α, OPh), 156·8 (s, C=O, carbamate), 167·2 (s, C=O, ester), 201·0 (s, C=S).

I.R. ν_{max}, 3220, 3300 (NH), 1765 (C=O, ester), 1695 (C=O, carbamate).

U.V. $\lambda_{max}(CHCl_3)$, 265 ($\varepsilon = 1{\cdot}6 \times 10^4$).

Mass (m/e), 264 (M-PhOH, 0·7), 156 (264-$PhCH_2OH$, 2·5), 94 (PhOH, 100).

Acc. mass: $C_{12}H_{12}N_2O_3S$ (M-PhOH) requires 264·0566, found 264·0557.

N-Benzyloxycarbonyl-thioalanyl-alanine methyl ester (55e)

A suspension of Z-Ala-Ala-OMe (55c) (250 mg, 0·8 mmol)[119] and Lawesson's reagent (25) (190 mg, 0;47 mmol) in dry toluene (20 ml) was heated at 85-95°C (2 h). The resulting solution was cooled to room temperature and the solvent evaporated. The oily residue was subjected to medium pressure column chromatography (10% EtOAc/DCM). The fractions containing the desired product (R_f 0·4) were combined and evaporated to give the title compound as a colourless, viscous oil (260 mg, 100%) that retained solvent traces tenaciously.

^{1}H n.m.r. (400 MHz), δ 1·45 and 1·46 (3H, d, 7 Hz, CH_3 $Alat^1$, Ala^2), 3·75 (3H, s, OCH_3), 4·60 (1H, m, CH Ala^2), 5·05 (1H, p, 7 Hz, CH $Alat^1$), 5·10 (2H, q, 13 Hz, OCH_2), 5·70 (1H, br d, NH $Alat^1$), 7·35 (5H, m, aromatic), 8·5 (1H, br s, NH Ala^2).

^{13}C n.m.r. δ 16·7 and 22·3 (q, CH_3 $Alat^1$, Ala^2), 52·4 (q, OCH_3), 53·4 (d, CH Ala^2), 56·3 (d, CH $Alat^1$), 67·1 (t, $PhCH_2O$), 127·8-128·5 (3C, aromatic), 136·2 (C_α, aromatic), 155·9 (s, C=O, carbamate), 172·2 (s, C=O, ester), 205·5 (s, C=S).

I.R. ν_{max} (Liq. film), 3300 (NH), 1700-1750 (broad, C=O, ester, carbamate).

U.V. λ_{max} ($CHCl_3$), 270 ($\varepsilon = 1{\cdot}09 \times 10^4$)

Mass (m/e) 324 (M^+, 6), 216 (M-$PhCH_2OH$, 22), 184 (216-MeOH, 10), 108 ($PhCH_2OH$, 43), 91 ($C_7H_7^+$, 100).

Acc. mass: $C_{15}H_{20}N_2O_4S$ requires 324·1139, found 324·1151.

N-Benzyloxycarbonyl-thioglycyl-glycine methyl ester (55a)

mp Table 5

^{1}H n.m.r. δ 3·75 (3H, s, OCH_3), 4·25 (2H, d, 7 Hz, CH_2 Gly^2), 4·4 (2H, d, 6 Hz, CH_2 Glyt^1), 5·1 (2H, s, $PhC\underline{H}_2O$), 5·9 (1H, br t, 6 Hz, NH Glyt^1), 7·3 (5H, s, aromatic), 8·6 (1H, br t, NH Gly^2).

^{13}C n.m.r. δ 46·7 (t, CH_2 Gly^2), 51·9 (q, OCH_3), 52·5 (t, CH_2 Glyt^1), 67·4 (t, $\underline{C}H_2O$), 128·0-128·6 (3C, aromatic), 136·1 (C_α, aromatic), 156·8 (s, C=O, carbamate), 168·8 (s, C=O, ester), 200·7 (s, C=S).

I.R. ν_{max}, 3300, 3400 (NH), 1750 (C=O, ester), 1710 (C=O, carbamate).

U.V. λ_{max}($CHCl_3$), 266 ($\varepsilon = 1{\cdot}27 \times 10^4$).

Mass (m/e), 296 (M^+, 28), 188 (M-$PhCH_2OH$, 3), 156 (188-MeOH, 2), 91 ($C_7H_7^+$, 100).

Acc. mass: $C_{13}H_{16}N_2O_4S$ requires 296·0827, found 296·0833.

N-Benzyloxycarbonyl-thioglycyl-glycine 4-methoxybenzyl ester (55b)

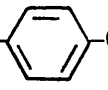
mp Table 5

^{1}H n.m.r. δ 3·75 (3H, s, OCH_3), 4·2 (2H, d, 6 Hz, CH_2 Glyt^1), 4·4 (2H, d, 5 Hz, CH_2 Gly^2), 5·1 (4H, s, $PhC\underline{H}_2O$, $ArC\underline{H}_2O$), 6·9 (2H, d, 9 Hz, (MeO)P$\underline{h}$), 7·0-7·4 (8H, aromatic (7H), NH Glyt^1), 9·6 (1H, br t, NH Gly^2). ($CDCl_3$/DMSO-d_6)

^{13}C n.m.r. δ 46·7 (t, CH_2 Gly^2), 51·6 (t, CH_2 Glyt^1), 55·1 (q, OCH_3), 66·6 and 66·7 (t, $Ar\underline{C}H_2O$), 113·9 (d, C_β, (MeO)Ph-), 128·1-130·0 (aromatic), 136·6 (s, $C_\alpha\,\underline{Ph}CH_2$), 156·7 (s, C_α, (MeO)Ph-), 159·7 (s, C=O, carbamate), 168·1 (s, C=O, ester), 201·3 (s, C=S). ($CDCl_3$/DMSO-d_6)

I.R. ν_{max}, 3350 (NH), 1740 (C=O, ester), 1700 (C=O, carbamate).

U.V. λ_{max}, 264 ($\varepsilon = 1{\cdot}23 \times 10^4$).

Mass (m/e), 402 (M^+, 1·4), 294 (M-$PhCH_2OH$, 2·5), 121 (MeO-  -CH_2^+, 100), 91 ($C_7H_7^+$, 55).

N-*tert*-Butoxycarbonyl-thioglycyl-glycine benzyl ester (55d)

mp Table 5

^{1}H n.m.r. δ 1·45 (9H, s, CH_3), 4·2 (2H, d, 5 Hz, CH_2 Glyt^1), 4·45 (2H, d, 5 Hz, CH_2 Gly^2), 5·2 (2H, s, Ph$\underline{CH_2}$O), 5·6 (1H, br t, NH Glyt^1), 7·35 (5H, s, aromatic), 8·8 (1H, br s, NH Gly^2).

^{13}C n.m.r. δ 28·3 (q, CH_3), 46·5 (t, CH_2 Gly^2), 52·1 (t, CH_2 Glyt^1), 67·5 (t, OCH_2), 80·8 (s, $(CH_3)_3$ $\underline{C}$-), 128·3-128·6 (aromatic), 135·1 (s, C_α aromatic), 156·3 (s, C=O, carbamate), 168·4 (s, C=O, ester), 201·0 (s, C=S).

I.R. ν_{max}, 3350 (NH), 1730 (C=O, ester), 1690 (C=O, carbamate.

U.V. λ_{max}($CHCl_3$), 266 (ε = 1·28 x 10^4).

Mass (m/e), 338 (M^+, 7), 282 (M-C_4H_8, 17), 265 (M-C_4H_9O, 5), 91 ($C_7H_7^+$, 100), 57 (C_4H_9, 42).

Acc. mass: $C_{16}H_{22}N_2O_4S$ requires 338·1295, found 338·1302.

N-Benzyloxycarbonyl-thioglycyl-phenylalanine methyl ester (23)[47]

mp Table 5

^{1}H n.m.r. δ 3·20 (2H, dd, 6 Hz, CH_2 Phe^2), 3·6 (3H, s, OCH_3), 4·1 (2H, d, 6 Hz, CH_2 Glyt^1), 5·0 (2H, s, OCH_2), 5·55 (1H, m, CHPhe^2), 6·0 (1H, t, 6 Hz, NH Glyt^1), 7·0-7·4 (10 H, s, m, aromatic), 9·6 (1H, br d, NH Phe^2).

^{13}C n.m.r. δ 36·3 (t, CH_2 Phe^2), 51·8 (t, CH_2 Glyt^1), 52·3 (q, OCH_3), 58·3 (d, CH Phe^2), 67·1 (t, OCH_2), 127·1-129·1 (aromatic C), 135·3 and 136·0 (C_α, aromatic), 156·6 (s, C=O, carbamate), 170·8 (s, C=O, ester), 199·9 (s, C=S).

I.R. ν_{max}, 3340 (NH), 1700-40 (C=O, ester, carbamate).

U.V. λ_{max}, 265 (ε = 1.34 x 10^4).

Mass (m/e), 386 (M^+, 11), 353 (M-$MeOH_2^+$, 3·5), 251 (M-Z, 5), 162 ($PhCH_2^+$=$CHCO_2Me$, 37), 91 ($C_7H_7^+$, 100).

Acc. mass: $C_{20}H_{22}N_2O_4S$, calc. 386·1295, found 386·1300.

N-Benzyloxycarbonyl-thiophenylalanyl-glycyl methyl ester (55f)

mp Table 5

^{1}H n.m.r. δ 3·1 (2H, dd, 2 Hz, 7 Hz, CH_2 Phet^1), 3·7 (3H, s, OCH_3), 4·2 (2H, d, 5 Hz, CH_2 Gly^2), 4·9 (1H, m, CH Phet^1), 5·0 (2H, s, $PhC\underline{H}_2O$), 5·8 (1H, d, 9 Hz, NH Phet^1), 7·2 (5H, s, aromatic), 7·3 (5H, s, aromatic), 8·35 (1H, br s, NH Gly^2).

^{13}C n.m.r. δ 42·2 (t, CH_2 Phet^1), 46·8 (t, CH_2 Gly^2), 52·3 (q, OCH_3), 62·6 (d, CH Phet^1), 67·1 (t, $PhC\underline{H}_2O$), 127·0–129·3 (aromatic), 136·3 and 136·5 (C_α, aromatic), 155·9 (s, C=O , carbamate), 168·5 (s, C=O, ester), 204·1 (s, C=S).

I.R. ν_{max}, 3325 (NH), 1750 (C=O, ester), 1695 (C=O, carbamate).

U.V. λ_{max}($CHCl_3$), 269 (ε = 1·23 x 10^4).

Mass (m/e), 386 (M^+, 0·7), 278 (M-$PhCH_2OH$, 17), 219 (278-CO, MeO, 12), 91 ($C_7H_7^+$, 100).

Acc. mass: $C_{20}H_{22}N_2O_4S$ requires 386·1295, found 386·1301.

N-Benzyloxycarbonyl-thio*iso*leucyl-glycine methyl ester (55g)

mp Table 5

^{1}H n.m.r. δ 0·9 and 0·95 (3H, s, CH_3 Ilet^1), 1·0–2·0 (3H, br m, $CH_3\underline{CH_2}CH(CH_3)$ Ilet^1), 3·7 (3H, s, OCH_3), 4·2–4·55 (3H, m, CH_2 Gly^2, CH Ilet^1) 5·1 (2H, s, $PhCH_2O$), 5·95 (1H, d, 9 Hz, NH Ilet^1), 7·3 (5H, s, aromatic), 9·2 (1H, br s, NH Gly^2).

^{13}C n.m.r. δ 11·0 and 15·4 (q, CH_3 Ilet^1), 24·8 (t, CH_2 Ilet^1), 39·8 (d, $\underline{CH}(CH_3)CH_2CH_3$), 46·6 (t, CH_2 Gly^2), 52·3 (q, OCH_3), 65·3 (d, CH, (i-Bu) Ilet^1), 67·0 (t, $PhC\underline{H}_2O$), 127·6–128·4 (aromatic), 136·3 (s, C_α, aromatic), 156·5 (s, C=O, carbamate), 168·6 (s, C=O, ester), 205·8 (s, C=S).

I.R. ν_{max} (Liq. film), 3300 (NH), 1700–1750 (C=O, ester, carbamate).

U.V. λ_{max} ($CHCl_3$), 269 (ε = 1·13 x 10^4).

Mass (m/e), 352 (M^+, 3), 296 (M-C_4H_8, 2), 220 (6),
188 (296-$PhCH_2OH$, 20), 176 (23), 91 ($C_7H_7^+$, 100).
Acc. mass: $C_{17}H_{24}N_2O_4S$ requires 352·1451, found 352·1459.

Thioglycyl-glycine methyl ester hydrobromide salt (61)

A mixture of Z-Glyt-Gly-OMe (55a) (640 mg, 2·2 mmol) and HBr/AcOH (45% w/v, 10 ml) was stirred at room temperature (1 h) in a flask protected from moisture ingress ($CaCl_2$). Dry ether (50 ml) was added and the white precipitate filtered off and washed with a small amount of ether (10 ml). This afforded the title compound as a white powder (500 mg, 95%).

mp 171·0–173·0°C (decomp.)

^{1}H n.m.r. δ 3·70 (3H, s, OCH_3), 3·90 (2H, br s, CH_2 Glyt^1), 4·40 (2H, d, 4 Hz, CH_2 Gly^2), 8·25 (3H, br s, NH_3^+), 11·0 (1H, br s, NH), (DMSO-d_6).

^{13}C n.m.r. δ 47·2 and 47·8 (t, CH_2 Glyt^1 Gly^2), 53·6 (q, OCH_3), 169·5 (s, C=O, ester), 198·0 (s, C=S), (DMSO-d_6).

I.R. ν_{max}, 3000 (NH_2, broad), 1745 (C=O, ester).

U.V. λ_{max} (H_2O), 262 ($\varepsilon = 1{\cdot}06 \times 10^4$).

Mass (m/e), 162 (M-HBr, 65), 145 (162-NH_3, 3). 133 (162-$CH_2\overset{+}{N}H$, 21), 30 ($CH_2\overset{+}{N}H_2$, 100).

Acc. mass: $C_5H_{10}N_2O_2S$ (M-HBr) requires 162·0461, found 162·0475.

N-Benzyloxycarbonyl-alanyl-thioglycyl-glycine methyl ester (63)

To a suspension of the hydrobromide (61) (242 mg, 1 mmol) and Z-Ala-OH (62) (223 mg, 1 mmol) in DCM (10 ml) at 0–5°C was added a solution of TEA (101 mg, 1 mmol) in DCM (5 ml) and the reaction stirred (0·1 h). A solution of DCC (206 mg, 1 mmol)[79] in DCM (10 ml) was added slowly dropwise. The reaction mixture was allowed to warm to room temperature and stirred overnight. The white precipitate (DCU) was

filtered off and the filtrate evaporated and subjected to medium pressure column chromatography (5% EtOH/$CHCl_3$). The fractions containing the desired product (R_f 0·35) were combined and evaporated to afford the title compound as a white, crystalline solid (290 mg, 79%).

mp 108-109°C

^{1}H n.m.r. δ 1·40 (3H, d, 7 Hz, CH_3 Ala^1), 3·70 (3H, s, OCH_3), 4·3 (5H, m, CH_2 Glyt^2, Gly^3, CH Ala^1), 5·05 (2H, s, Ph$\underline{CH_2}$O), 5·90 (1H, d, 6 Hz, NH Ala^1), 7·3 (5H, s, aromatic), 7·55 (1H, br t, NH, Glyt^2), 9·00 (1H, (br t), NH, Gly^3).

^{13}C n.m.r. δ 18·2 (q, CH_3 Ala^1), 46·9 (t, CH_2 Gly^3), 50·1 (t, CH_2 Glyt^2), 51·2 (d, CH Ala^1), 52·5 (q, CH_3O), 67·2 (t, Ph$\underline{CH_2}$O), 128·0-128·6 (aromatic, 3C), 136·2 (C_α, aromatic), 156·5 (s, C=O, carbamate), 169·0 (s, C=O, ester), 173·6 (s, C=O, amide), 200·3 (s, C=S).

I.R. ν_{max}, 3300 (NH), 1750 (C=O, ester), 1700 (C=O, carbamate), 1670 (C=O, amide).

U.V. λ_{max} ($CHCl_3$), 267 ($\varepsilon = 1{\cdot}15 \times 10^4$).

Mass (m/e), 367(M^+, 32), 259 (M-$PhCH_2OH$, 14), 161 (Glyt-Gly-OMe, 12), 91 ($C_7H_7^+$, 100).

Acc. mass: $C_{16}H_{21}N_3O_5S$ requires 367·1197, found 367·1215.

Thioglycyl-glycine benzyl ester trifluoroacetate salt (64)

A solution of BOC-Glyt-Gly-OBzl (55b) (169 mg, 0·5 mmol) in aqueous TFA (90% v/v, 10 ml) was stirred at room temperature (0·5 h). The solvent was evaporated and toluene (2 x 20 ml) used (azeotrope formation) to aid the removal. This gave a colourless, viscous oil which crystallized on standing to afford the title compound as a white solid (140 mg, 94%).

mp 129-131°C (decomp.)

^{1}H n.m.r. δ 3·95 (3H, s, CH_2 Glyt^1), 4·50 (2H, s, CH_2 Gly^2), 5·20 (2H, s, PhC$\underline{H}_2$O), 7·40 (5H, s, aromatic), 8·9 (3H, v br s, $\overset{+}{N}H_3$). (DMSO-d_6).

^{13}C n.m.r. δ 45·8 (t, CH_2 Glyt^1), 46·5 (t, CH_2 Gly^2), 66·1 (t, $\underline{C}H_2$Ph), 127·9-128·3 (3C, aromatic), 135·6 (C_α, aromatic), 167·5 (s, C=O, ester), 196·8 (s, C=S). (DMSO-d_6).

I.R. ν_{max}, 3200 (NH), 1760 (C=O, ester), 1690 (C=O, TFA).

U.V. λ_{max} (H_2O), 262 (ε = 1·07 x 10^4).

Mass (m/e), 238 (M^+,TFA 17), 221 (238-NH_3, 2), 209 ($\overset{+}{N}H_2CH$ loss, 20), 147 (238-$C_7H_7^+$, 6), 130 (238-$PhCH_2OH$, 6).

Acc. mass: $C_{11}H_{14}N_2O_2S$ (M-TFA) requires 238·0773, found 238·0769.

Thio*iso*leucyl-glycine hydrobromide salt (65)

A solution of Z-Ilet-Gly-OMe (55g) (200 mg, 0.57 mmol) in HBr/AcOH (48% w/v, 5 ml), protected from moisture ingress ($CaCl_2$), was stirred at room temperature (1·5 h). Ether (50 ml) was added and the whole evaporated. The slightly orange oily residue was evaporated from DCM (5 x 5 ml) giving an orange solid. After washing with ether (10 ml) the title compound was obtained as a pale orange powder (75 mg, 83%).

mp 175-179°C (decomp.)

^{1}H n.m.r. δ 0·90 (3H, t, 7 Hz, $C\underline{H}_3CH_2CH$), 0·95 (3H, d, 6 Hz, $C\underline{H}_3CH$), 1·0-2·1 (3H, m, $CH_3C\underline{H}_2\underline{C}H$), 4·05 (1H, d, 7 Hz, CH Ilet^1), 4·45 (2H, s, CH_2 Gly^2).

^{13}C n.m.r. δ 0·8 and 14·9 (q, CH_3 Ilet^1), 24·8 (t, CH_2 Ilet^1), 39·1 (d, $\underline{C}HCH_2CH_3$), 47·6 (t, CH_2 Gly^2), 63·5 (d, $\underline{C}H$ C(S)), 172·1 (s, C=O, acid), 200·8 (s, C=S).

Thioglycyl-glycine hydrobromide salt (66)

A solution of Z-Glyt-Gly-OMe (55a) (190 mg, 0·64 mmol) in HBr/AcOH (48% w/v, 10 ml) was heated at 55°C (6 h). The reaction mixture was cooled to room temperature and ether (50 ml) added. The precipitated white solid was filtered and washed with a small amount of ether (<10 ml). This gave the title compound (140 mg, 95%) as a cream powder.

mp 91-94°C

^{1}H n.m.r. δ 3·9 (2H, s, CH_2), 4·1 (2H, s, CH_2), 4·75 (s, HDO).

I.R. ν_{max}, 2800 (OH, broad), 1615 (C=O, acid).

U.V. λ_{max} (H_2O), 259

Mass (m/e), 148 (M^+-HBr, 0·5), 130 (M^+-HBr-H_2O, 7), 114 (130-NH_2, 47), 80, 82 (HBr, 50 each), 30 (100).

Acc. mass: $C_4H_6N_2OS$ (M-HBr-H_2O) requires 130·0200, found 130·0201.

Thioglycyl-phenylalanine methyl ester hydrobromide salt (68)

A solution of Z-Glyt-Phe-OMe (23) (2·07 g, 5·4 mmol)[47] in AcOH (glacial, 2 ml) was treated with HBr/AcOH (48% w/v, 8 ml) and the solution stirred at room temperature (0·5 h). Ether (50 ml) was added and the precipitate filtered off and washed with ether (2 x 20 ml). This afforded the title compound as a white powder (1·41 g, 79%), clearly homogeneous by TLC (5% MeOH/$CHCl_3$, R_f 0·5, UV, Ninhydrin yellow), which was used immediately.

^{1}H n.m.r. (60 MHz), δ 3·2 (2H, dd, 2Hz, 7Hz, CH_2 Phe^2), 3·65 (3H, s, OCH_3), 3·9 (2H, s, CH_2 Glyt^1), 4·6 (HDO), 5·2 (1H, t, 7Hz, CH Phe^2), 7·2 (5H, s, aromatic).

Mass (m/e), 252 (M-HBr, 10), 235 (252-NH_3, 48), 203 (235-MeOH, 41), 162 (252-$C_7H_6^+$, 33), 91 ($C_7H_7^+$, 100).

N-Benzyloxycarbonyl-glycyl-thioglycyl-phenylalanine methyl ester (71)

To a suspension of Z-Gly-OH (69) (847 mg, 4 mmol) in DCM (80 ml) at -20°C was added DPPCl (70) (1000 mg, 4·2 mmol) and NMM (0·45 ml, 4·1 mmol) and the mixture stirred (0·25 h). The hydrobromide (68) (1·349 g, 4·1 mmol) and NMM (0·55 ml, 5 mmol) were added and the reaction allowed to reach room temperature and stirred overnight. The reaction mixture was evaporated and the crude residue treated with EtOAc (50 ml). The resultant suspension was washed with aqueous sodium bicarbonate solution (5%, 2 x 25 ml). The organic phase was separated and washed further with aqueous citric acid (5%, 2 x 25 ml) and water (2 x 25 ml). The organic layer was dried ($MgSO_4$), filtered and evaporated.†

The orange oil thus obtained was subjected to medium pressure column chromatography (25% EtOAc/DCM). The fractions containing the desired product were combined and evaporated to give the title compound as a white solid (1·2 g, 67%).

mp 54·5-56°C

^{1}H n.m.r. δ 3·25 (2H, dd, 5·5 Hz, 5·5 Hz, CH_2 Phe^3), 3·65 (3H, s, OCH_3), 3·8 (2H, d, 6Hz, CH_2 Gly^1), 4·2 (2H, m, CH_2 Glyt^2), 5·10 (2H, s, Ph$\underline{CH_2}$O), 5·35 (1H, m, CH Phe^3), 5·9 (1H, t, 5Hz, NH Gly^1), 7·1-7·4 (10H, m, s, aromatic), 7·5 (1H, t, NH Glyt^2), 8·95 (1H, d, 7 Hz, NH Phe^3).

^{13}C n.m.r. δ 36·6 (t, CH_2 Phe^3), 44·6 (t, CH_2 Gly^1), 49·9 (t, CH_2 Glyt^2), 52·4 (q, OCH_3), 58·9 (d, CH Phe^3), 67·3 (t, Ph$\underline{CH_2}$O), 127·2-136·2 (aromatic), 156·7 (s, C=O, carbamate), 170·0 (s, C=O, ester), 171·0 (s, C=O, amide), 199·5 (s, C=S).

†Unless stated otherwise, all peptide coupling reactions were worked up in this standard fashion.

I.R. ν_{max}, 3350 (NH), 1740 (C=O, ester), 1680 (C=O, carbamate), 1660 (C=O, amide).

U.V. λ_{max}, 265 (ε = 1·22 x 10^4).

Mass (m/e), 443 (M^+, 3), 411 (M-MeOH, 1·5), 335 (M-$PhCH_2OH$, 11), 303 (335-MeOH, 6·5), 91 ($C_7H_7^+$, 100).

Elemental Analysis, Found: C, 59·46; H, 5·86; N, 9·60; S, 7·14.

Calc. for $C_{22}H_{25}N_3O_5S$: C, 59·6; H, 5·64; N, 9·48; S, 7·22.

N-Benzyloxycarbonyl-thioglycyl-glycine (13a)[35]

To a solution of Z-Glyt-Gly-OMe (55a) (140 mg, 0·47 mmol) in acetone (8 ml) was added a solution of sodium hydroxide (0·25 M, 2 ml) and the reaction stirred at room temperature (18 h). A further aliquot of sodium hydroxide solution (3 drops) was added and the coloured (red) solution stirred (1 h). The reaction mixture was partially evaporated, to remove acetone, and water (23 ml) added to the liquid residue. The aqueous solution was extracted with ether (25 ml, 10 ml) and then acidified (2 M, HCl) to pH 1. The aqueous solution was again extracted, with EtOAc (2 x 25 ml), and the organic phase dried ($MgSO_4$), filtered and evaporated affording a slightly coloured (orange) solid (72 mg, 55%) consisting of predominantly the title compound.

^{1}H n.m.r.† δ, 4·2 (2H, d, 6 Hz, CH_2 Gly^2), 4·3 (2H, d, 5 Hz, CH_2 $Glyt^1$), 5·10 (2H, s, $C\underline{H_2}O$), 7·10 (1H, br s, NH Gly^1), 7·35 (5H, s, aromatic), 9·4 (1H, br t, NH Gly^2). (20% DMSO-d_6/$CDCl_3$).

I.R. ν_{max}, 2900 (OH), 1730 (C=O, carbamate), 1700 (C=O, acid).

Mass (m/e), 282 (M^+, 2), 264 (M-H_2O, 1·25), 91 ($C_7H_7^+$, 100).

†Other (minor) signals seen but unassigned.

N-Benzyloxycarbonyl-glycyl-thioglycyl-phenylalanine (77)

To a solution of Z-Gly-Glyt-Phe-OMe (71) (150 mg, 0·34 mmol) in THF (9 ml) was added a solution of sodium hydroxide (0·39 M, 0·9 ml) and the mixture stirred at room temperature (18 h). After this time some of the ester (71) still remained. Additional sodium hydroxide solution (0·5 ml, total 1·6 equiv.) was added and the mixture stirred for a further period (5·5 h). The solution was partially evaporated to remove THF and the residual liquid partitioned between water (10 ml) and ether (10 ml). The aqueous layer was washed with a further ether aliquot (5 ml) and then acidified (pH 1-2) with hydrochloric acid (2 M). Extraction with EtOAc (3 x 10 ml), drying of the organic phase ($MgSO_4$), filtration and evaporation gave a slightly coloured (yellow) oil (130 mg) which comprised predominantly the title compound by TLC (10% MeOH/$CHCl_3$, 1% AcOH; R_f 0·4) and ^1H n.m.r. (60 MHz).

^1H n.m.r.† (60 MHz) δ 3·2 (2H, m, CH_2 Phe^3), 3·8 and 4·2 (2H, m, CH_2 Gly^1, $Glyt^2$), 5·0 (1H, s, CH_2O), 5·3 (1H, m, CH Phe^3) 7·1 and 7·2 (5H, s, aromatic), 7·8 (1H, br t, NH Phe^3), 9·0 (1H, br d, NH $Glyt^2$) ($CDCl_3$/d_6-acetone).

Attempted preparation of Glycyl-thioglycyl-phenylalanine methyl ester hydrobromide salt (78)

To a solution of Z-Gly-Glyt-Phe-OMe (71) (40 mg, 0·09 mmol) in acetic acid (1 ml) was added a solution of HBr/AcOH (48% w/v, 0·5 ml) and the reaction stirred at room temperature (0·5 h). Ether was

†This sample was not homogeneous as evidenced by an additional broad singlet at 6·9 ppm, the low integral value for the singlet at 5·0 ppm and TLC. However, purification by medium pressure column chromatography (4% MeOH/$CHCl_3$, 1% AcOH) was feasible giving a moderate yield of the title compound (60%) as an off-white foam.

added and the whole extracted with water (25 ml). The aqueous layer was evaporated to afford a coloured (orange) semi-crystalline solid. TLC analysis of this material and ^{1}H n.m.r. (60 MHz) identified it probably as the doubly deprotected tripeptide, glycyl-thioglycyl-phenylalanine hydrobromide salt (78) although a rigorous characterization was not carried out.

^{1}H n.m.r. (60 MHz) δ 3·0 (2H, m, CH_2 Phe^3), 3·7 and 3·8 (4H, 2s, CH_2 Gly^1, Glyt^2), 4·2 (1H, m, CH Phe^3), 7·1 (5H, s, aromatic), (all other protons exchanged with solvent).

Attempted preparation of N-Benzyloxycarbonyl-thioglycyl-glycine 4-nitrophenyl ester (81)

A suspension of Z-Gly-Gly-ONp (80) (250 mg, 0·65 mmol)[120] and Lawesson's reagent (25) (144 mg, 0·36 mmol) in dry toluene (10 ml) was heated at 80°C (0·25 h). The resultant yellow solution was cooled to room temperature. TLC analysis of the mixture (50% EtOAC/DCM) showed that no starting material remained and a possible product (R_f 0·7, UV, I_2 orange) had formed. This material was not stable to medium pressure column chromatography with silica although some fractions were combined and evaporated to give a small amount of para-nitrophenol (82) (identified by 60 MHz ^{1}H n.m.r.).[121]

N-Benzyloxycarbonyl-thioglycyl-glycyl-glycine methyl ester (84)

A mixture of Z-Glyt-Gly-OPh (55c) (171 mg, 0·5 mmol), glycine methyl ester hydrochloride (85) (63 mg, 0·5 mmol) and TEA (51 mg, 0·5 mmol) in IPA (25 ml) was refluxed (8·5 h). The resulting solution was cooled to room temperature, the solvent evaporated and the crude residue subjected to medium pressure column chromatography (30% EtOAc/DCM). The fractions containing the desired product (R_f

0·15) were combined and evaporated. This gave the title compound as a white solid (83 mg, 47%).

mp 130·5-132·5°C

^{1}H n.m.r. δ 3·70 (3H, s, OCH_3), 3·90 (2H, d, 6 Hz, CH_2 Gly^3), 4·20 (2H, d, 6 Hz, CH_2 $Glyt^1$), 4·30 (2H, d, 5 Hz, CH_2 Gly^2), 5·10 (2H, s, $PhCH_2O$), 7·3 (6H, s, aromatic , NH $Glyt^1$), 8·25 (1H, br t, NH Gly^3), 9·60 (1H, br t, NH Gly^2). ($CDCl_3$/DMSO-d_6).

^{13}C n.m.r. δ 40·9 (t, CH_2 Gly^2), 48·1 (t, CH_2 Gly^1), 52·0 (q, t, CH_2 Gly^3, OCH_3), 66·7 (t, $PhCH_2O$), 127·9-129·0 (3C, aromatic), 136·5 (s, C_α, aromatic), 156·7 (s, C=O, carbamate), 167·8 (s, C=O, ester), 169·9 (s, C=O, amide), 200·2 (s, C=S). ($CDCl_3$/DMSO-d_6).

I.R. ν_{max}, 3300 (NH), 1745 (C=O, ester), 1700 (C=O, carbamate), 1665 (C=O, amide).

U.V. λ_{max} ($CHCl_3$), 267 ($\varepsilon = 1{\cdot}1 \times 10^4$).

Mass (m/e), 353 (M^+, 5), 319 (M-MeOH, 3), 245 (M-$PhCH_2OH$, 29), 108 ($PhCH_2OH$, 50), 91 ($C_7H_7^+$, 100).

Acc. mass: $C_{15}H_{19}N_3O_5S$ requires 353·1041, found 353·1044.

N-Benzyloxycarbonyl-thioglycyl-glycyl-phenylalanyl-leucine methyl ester (86)

A solution of Z-Glyt-Gly-OPh (55c) (700 mg, 1·96 mmol), phenylalanyl-leucine methyl ester hydrochloride salt (88) (808 mg, 2·46 mmol) and TEA (278 mg, 2·75 mmol) in IPA (50 ml) was heated to reflux (2·5 h). The reaction mixture was cooled to room temperature and the solvent evaporated to give a dark residue. The crude material was washed in the usual way and subjected to medium pressure column chromatography (30% EtOAC/DCM). The fractions containing the desired product (R_f 0·2) were combined and evaporated to afford the title compound.(420 mg, 43% (corrected)) as a white solid after trituration

with a small amount of petroleum ether (40-60).

mp 110-111·0°C

^{1}H n.m.r. (400 MHz) δ 0·87 and 0·88 (3H, d, 6Hz, CH_3 Leu^4), 1·45-1·63 (3H, m, $CH_2CH(CH_3)_2$), 2·95 (1H, dd, 7·5 Hz, 14 Hz, CH_2 Phe^3), 3·07 (1H, dd, 6 Hz, 14 Hz, CH_2 Phe^3), 3·70 (3H, s, OCH_3), 4·15-4·42 (4H, m, CH_2 Glyt^1, Gly^2), 4·55 (1H, m, CH-$CH_2CH(CH_3)_2$), 4·90 (1H, m, CH Phe^3), 5·11 (2H, q, 12 Hz, $PhCH_2O$), 6·10 (1H, br t, NH Glyt^1), 7·1-7·35 (12H, complex, Ar, 2NH Phe^3, Leu^4), 9·05 (1H, br s, NH Gly^2).

^{13}C n.m.r. δ 21·3 and 22·7 (q, CH_3 Leu^4), 24·2 (d, $CH(CH_3)_2$ Leu^4), 40·3 (t, CH_2 Leu^4, CH_2 Phe^3), 47·6 (t, CH_2, Gly^2), 50·4 and 51·3 (d, CH Leu^4, CH Phe^3), 51·7 (t, CH_2 Glyt^1), 53·7 (q, OCH_3), 65·8 (t, $PhCH_2O$), 126·2-129·1 (aromatic), 136·8 and 137·6 (C_α, aromatic), 156·0 (s, C=O, carbamate), 166·7 (s, C=O, ester), 171·0 and 172·6 (s, C=O, amide), 200·1 (s, C=S) (DMSO-d_6).

I.R. ν_{max}, 3320 (NH), 1740 (C=O, ester), 1640 (C=O, amide).

U.V. λ_{max} 264 ($\varepsilon = 8·79 \times 10^3$).

Mass (m/e) (FAB +), 557 (M+1, 11), 525 (M-MeOH, 3). 293 (H_2-Phe-Leu-OMe^+, 51), 146 (H_2-Leu-OMe^+, 90), 120 ($PhCH_2CHNH_3^+$, 100).

Thioglycyl-glycyl-phenylalanyl-leucine methyl ester (90)

To a solution of Z-Glyt-Gly-Phe-Leu-OMe (86) (387 mg, 0·7 mmol) in AcOH (1·5 ml) protected from moisture ingress ($CaCl_2$) was added a solution of HBr/AcOH (48% w/v, 1 ml) and the reaction mixture stirred at room temperature (0·5 h). The mixture was diluted with ether (10 ml) and extracted into water (30 ml). The aqueous phase comprised two main components by TLC (5% MeOH/$CHCl_3$) at R_f 0·6 (weakly UV active, Ninhydrin purple) and R_f 0·3 (UV, Ninhydrin yellow). This mixture was basified (xs TEA) and extracted into EtOAc (5 x 10 ml). The organic phase was dried ($MgSO_4$), filtered and evaporated. The crude residue

was subjected to medium pressure column chromatography (5% MeOH/$CHCl_3$).

The fractions containing mainly the higher R_f product were combined and evaporated to give a coloured (yellow) oil which solidified in air and was identified from this and subsequent experiments as H-Phe-Leu-OMe (88) (48 mg, 23%).

^{1}H n.m.r. (60 MHz)† δ 0·9 (6H, d, 4 Hz, CH_3 Leu^2), 1·5 (3H, m, $C\underline{H}_2C\underline{H}(CH_3)_2$), 3·0 (2H, m, CH_2 Phe^1), 3·6 (3H, s, OCH_3), 4-4·5 (2H, m, CH Phe^1, CH Leu^2), 7·0 (5H, s, aromatic), 7·4 (1H, br d, NH Phe^1).

Mass (m/e), 260 (M^+-MeOH, 12), 204 (260-C_4H_8, 14), 120 ($\overset{+}{H_2}N{=}CHCH_2Ph$, 23), 91 ($C_7H_7^+$, 62).

The fractions containing the lower R_f material were treated in the same manner giving a colourless oil (140 mg, 47%) identified, tentatively at this stage, as the title compound (90).

^{1}H n.m.r. (60 MHz)† δ 0·9 (6H, d, 4Hz, CH_3 Leu^4), 1·5 (3H, br m, $C\underline{H}_2C\underline{H}(CH_3)_2$ Leu^4), 3·0 (2H, d, 6 Hz, CH_2 Phe^3), 3·55 (5H, s, OCH_3, MeOH), 3·8-4·8 (7H, m, CH_2 Glyt^1, Gly^2, CH Phe^3, CH Leu^4, NH), 6·9 (6H, s, aromatic, NH), 7·4 (1H, d, 8 Hz, NH).

Mass (m/e) (CI), 391 (M^+-Z-MeOH, 3), 301 (391-$C_7H_6^+$, 7), 261 (H-Phe-Leu$^+$, 95).

N-Benzyloxycarbonyl-O-benzyl-tyrosyl-thioglycyl-glycl-phenylalanyl-leucine methyl ester (87), [4+1] route

To a solution of Z-Tyr(Bzl)-OH (89) (118 mg, 0·3 mmol) in DCM (10 ml) at -20°C was added DPPCl (70) (69 mg, 0·3 mmol) and NMM (39 mg, 0·39 mmol). The reaction mixture was stirred (0·3 h) and a solution of the tetrapeptide (90) (122 mg, 0·29 mmol) in DCM (5 ml) added. The

†These samples gave broader resonances in their n.m.r. spectra than was usually observed.

reaction was allowed to attain room temperature and stirred overnight. The solvent was evaporated and the crude residue partitioned between EtOAc (25 ml) and aqueous $NaHCO_3$ (5% w/v, 15 ml). The organic phase was washed with further $NaHCO_3$ (15 ml), followed by aq. HCl (1·4 M, 2 x 15 ml). After drying ($MgSO_4$), filtration and evaporation the crude material was subjected to medium pressure column chromatography (2·5% MeOH/$CHCl_3$). Fractions containing the desired product (R_f 0·35) were combined and evaporated to afford the title compound as a white solid (130 mg, 56%) after trituration with a small amount of ether.

mp 162·5–164°C (decomp.)

^{1}H n.m.r. (400 MHz) δ 0·91 (3H, d, 7 Hz), 0·94 (3H, d, 7 Hz), 1·44–1·66 (3H, m), 2·68 (1H, dd, 10 Hz, 14 Hz), 2·75 (1H, dd, 11 Hz, 14 Hz), 3·04 (2H, dd, 3 Hz, 14 Hz), 3·60 (3H, s), 4·05 (2H, dd, 5·5 Hz, 18 Hz), 4·15 (2H, dd, almost coincident, 5·5 Hz, 18 Hz), 4·21 (1H, m), 4·27 (1H, m), 4·59 (1H, t of d), 4·91 (2H, q, 16 Hz, 2 Hz), 5·05 (2H, s), 6·88 (2H, d, 8 Hz), 7·18 (2H, d, 8 Hz), 7·2–7·5 (15H, m), 7·55 (1H, d, 8 Hz), 8·35 (1H, d, 10 Hz), 8·38 (1H, d, 8 Hz), 8·54 (1H, t, 5 Hz), 9·67 (1H, br t). (Assignments see Figure 5).

I.R. ν_{max}, 3325 (NH), 1740 (C=O, ester), 1700 (C=O, carbamate), 1655 (C=O, amide).

U.V. λ_{max}, 267 ($\varepsilon = 1{\cdot}05 \times 10^4$).

Mass (m/e) (FAB +), 810 ($M^+ + 1$, 8), 676 (M–Z, 12), 120 ($H_2N^+{=}CHCH_2Ph$, 100).

Elemental Analysis, Found: C, 65·08; H, 6·49; N, 8·56; S, 4·18.
Calc. for $C_{44}H_{51}N_5O_8S$: C, 65·24; H, 6·35; N, 8·65; S, 3·95.

Thioglycyl-glycine phenyl ester hydrobromide salt (91)

A solution of HBr/AcOH (48% w/v, 6 ml) was added to

Z-Glyt-Gly-OPh (55c) (535 mg, 1·5 mmol) in a flask fitted with a drying tube ($CaCl_2$). The resultant solution was stirred at room temperature (0·5 h). Ether (25 ml) was added and the precipitate filtered off and washed with a small amount of ether (<10 ml). After drying in air the title compound was obtained as a cream powder (439 mg, 96%) clearly homogeneous by TLC (5% MeOH/$CHCl_3$) (R_f 0·25, Ninhydrin yellow).

mp >200°C (decomp.)

^{1}H n.m.r. δ 4·60 (4H, m, CH_2), 7·0–7·4 (5H, m, OPh), 8·5 (3H, br s, $\overset{+}{N}H_3$), 11·10 (1H, br t, NH). ($CDCl_3$/DMSO-d_6).

I.R. ν_{max}, 3000 (NH_2, broad), 1760 (C=O, ester).

U.V. λ_{max} (H_2O), 262 (ε = 1·16 x 10^4).

N-Benzyloxycarbonyl-O-benzyl-tyrosyl-thioglycyl-glycine phenyl ester

(92)

To a solution of Z-Tyr(Bzl)-OH (89) (582 mg, 1·4 mmol) in DCM (20 ml) at -20°C was added DPPCl (70) (343 mg, 1·45 mmol) and NMM (145 mg, 1·45 mmol) and the mixture stirred (0·25 h). The hydrobromide (91) (439 mg, 1·3 mmol) and additional NMM (145 mg, 1·45 mmol) were added. The reaction was allowed to reach room temperature and then stirred overnight. After evaporation of solvent the crude residue was washed in the usual way. Drying of the organic phase ($MgSO_4$), filtration and evaporation gave an oily residue. Trituration with petroleum ether (60–80) afforded the title compound as an essentially pure cream solid (815 mg, 92%). Additional purification was achieved by medium pressure column chromatography (15% EtOAc/DCM) (R_f 0·25) to give a white solid.

mp 141–142°C (decomp.)

^{1}H n.m.r. δ 3·0 (2H, m, CH_2 Tyr^1), 4·3 (3H, br m, CH_2 Gly^3, CH Tyr^1),

4·5 (2H, br m, CH_2 Glyt^2), 5·0 (4H, s, CH_2O), 6·8-7·4 (20H, s, m, Ar, NH Tyr^1), 8·4 (1H, br t, NH Glyt^2), 9·7 (1H, br t, NH Gly^3) ($CDCl_3$/DMSO-d_6).

^{13}C n.m.r. δ 45·1, 47·9 and 49·8 (t, CH_2, Glyt^2, Gly^3, Tyr^1), 56·8 (d, CH Tyr^1), 66·3 and 69·7 (t, CH_2O), 114·6 (C_β, Tyr^1), 121·4 (C_β, OPh), 125·9-130·3 (aromatic), 136·5 and 137·0 (s, C_α, Z, Bzl), 150·4 and 156·5 (s, C_α, Tyr^1, OPh), 157·3 (s, C=O, carbamate), 166·5 (s, C=O, ester), 172·1 (s, C=O, amide), 201·4 (s, C=S). ($CDCl_3$/DMSO-d_6).

I.R. ν_{max}, 3300 (NH), 1760 (C=O, ester), 1690 (C=O, carbamate), 1670 (C=O, amide).

U.V. λ_{max}, 264 ($\varepsilon = 8{\cdot}15 \times 10^3$).

Mass (m/e),(FAB +), 612 (M+H).

Elemental Analysis, Found: C, 66·75; H, 5·40; N, 6·87; S, 5·23.

Calc. for $C_{34}H_{33}N_3O_6S$: C, 66·78; H, 5·51; N, 6·70; S, 5·15.

N-Benzyloxycarbonyl-O-benzyl-tyrosyl-thioglycyl-glycyl-phenylalanyl-leucine methyl ester (87), [3+2] route

A mixture of Z-Tyr(Bzl)-Glyt-Gly-OPh (92) (800 mg, 1·3 mmol), phenylalanyl-leucine methyl ester hydrochloride salt (88) (500 mg, 1·5 mmol) and TEA (328 mg, 3·25 mmol) in IPA (60 ml) was refluxed (2 h). The reaction was cooled to room temperature and evaporated before washing in the usual way. The organic phase, after drying ($MgSO_4$), filtration and evaporation was subjected to medium pressure column chromatography (50% EtOAc/DCM). Fractions containing the title compound (R_f 0·25) were combined and evaporated giving a slightly coloured solid. Trituration with ether (10 ml) afforded the title compound as a white solid (300 mg, 29%) identical to that prepared by the [4+1] route (TLC, ^{1}H n.m.r. (60 MHz), MS).

Reaction of Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87) with Trifluoroacetic acid (TFA)[80]

A solution of the protected pentapeptide (87) (40 mg, 0·05 mmol) in TFA (0·5 ml) was kept at room temperature in an n.m.r. sample tube (26 h). ^{1}H n.m.r. (60 MHz) after this time was not conclusive of any change. However, TLC (5% MeOH/$CHCl_3$, NH_3) evidence showed that no further starting material remained. The solvent was evaporated and the coloured (orange) oil partitioned between aqueous $NaHCO_3$ solution (5%, 10 ml) and ether (10 ml). The aqueous phase was washed with a further ether aliquot (10 ml). The organic phase contained a baseline component (some streaking) plus a material which was identical by TLC (R_f 0·7, Ninhydrin purple) to the dipeptide H-Phe-Leu-OMe (88). This experiment was not pursued further.

Reaction of Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87) with TFA/TFMSA[93]

A solution of the protected pentapeptide (87) (72 mg, 0·09 mmol) in TFA (3 ml) was treated with anisole (0·1 ml, 0·9 mmol) and then TFMSA (250 mg, 1·7 mmol) and the resultant coloured (red) solution stirred at room temperature (0·75 h). Water (2 ml) was added and the mixture partially evaporated. The residue was partitioned between water (20 ml) and EtOAc (20 ml). The organic phase was washed with a further water aliquot (10 ml) and then dried ($MgSO_4$), filtered and evaporated. The aqueous phase was also evaporated. The ^{1}H n.m.r. spectrum showed no organic materials present. The residue from the organic layer appeared to be more than one component by TLC. The ^{1}H n.m.r. spectrum showed the absence of the characteristic thioamide NH proton at 8-9 ppm. In view of these unpromising results no further action was taken.

Reaction of Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87) with Boron tribromide[94]

A cooled (-10°C) solution of the pentapeptide (87) (56 mg, 0·07 mmol) in a mixture of DCM (1 ml) and dimethylacetamide (1·5 ml) was treated with a solution of boron tribromide in DCM (1·0 M, 0·35 ml, 0·35 mmol). The reaction mixture was warmed to room temperature (1 h) and stirred (3 h). 'Wet' methanol (3 ml) was added and the solution evaporated. After further evaporations from methanol (2 x 3 ml) an oily residue was obtained which consisted predominantly of starting material by TLC (5% MeOH/$CHCl_3$, NH_3).

Reaction of Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87) with Boron tribromide and TFA[95]

A suspension of the pentapeptide (87) (52 mg, 0·06 mmol) in dry DCM (10 ml) was treated with the minimum amount of TFA (~0·1 ml) necessary to dissolve the substrate and the solution cooled (-10°C). A solution of boron tribromide in DCM (1·0 M, 0·5 ml, 0·5 mmol) was added and the mixture stirred at -10°C (1 h) and then at room temperature (1·5 h). Water (5 ml) was added slowly and the solution evaporated from methanol (3 x 5 ml) to remove any boron compounds present.

The coloured (orange) residue was taken into water (25 ml) once more and washed with EtOAc (25 ml). After evaporation the aqueous residue (four components by reverse phase TLC, 50:50, MeOH:water) was treated with an aqueous solution of TEA (0·7%, 1 ml) and water (20 ml). However, extraction with EtOAc (25 ml, 10 ml, 5 ml) was unsuccessful, so the basic water layer was re-evaporated to a small volume (1 ml) and the four components separated by descending preparative paper chromatography (Whatman 3M; 70:20:10, 1-Butanol:water:AcOH).

After extraction with ethanol (50 ml) the major component was obtained as a gum (5 mg).

Mass (m/e) (CI), No signals above 500 mass, 261 (H-Phe-Leu-OH-H_2O, 100).

Alkaline hydrolysis of Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87)

A solution of the pentapeptide (87) (50 mg, 0·062 mmol) in THF (2·5 ml) was treated with aqueous sodium hydroxide (0·135 M, 0·5 ml, 0·675 mmol) and the mixture stirred (4 d). After this time substantial amounts of starting material (87) still remained. The solvent was partially evaporated to remove THF and partitioned between water (20 ml) and EtOAc (25 ml). The organic phase was separated, dried ($MgSO_4$), filtered and evaporated. The crude residue, which consisted of starting material (87) plus three other components by TLC (10% MeOH/$CHCl_3$, 1% AcOH), was dissolved in a small amount of EtOAc (2-3 ml) and applied to a preparative TLC plate. Elution of the plate (10% MeOH/$CHCl_3$, 1% AcOH), removal of the four component sections and extraction with THF gave recovered pentapeptide (87) (25 mg, 0·031 mmol) and the other main product (R_f 0·25) as a coloured oil (9 mg). The other two components were not pursued.

Mass (m/e) (CI), 444 (7·6), 437 (7·8), 369 (6·8), 305 (6·4), 285 (7·4), 219 (100).

Attempted hydrogenation[97] of Z-Tyr(Bzl)-Glyt-Gly-Phe-Leu-OMe (87)

To a suspension of the pentapeptide (87) (110 mg, 0·14 mmol) in methanol (4 ml) was added AcOH (5 ml). The resulting solution was treated with ammonium formate (34 mg, 0·54 mmol) and 10% Pd on charcoal (50 mg) and stirred at room temperature (1 h). TLC (5% EtOAc/$CHCl_3$, NH_3) showed that little or no reaction had occurred. Recovery of starting material was achieved by filtration through a

pad of Celite followed by evaporation of the filtrate. The residue was taken into EtOAC (60 ml) and washed with saturated brine (2 x 20 ml). The organic phase was dried ($MgSO_4$), filtered and evaporated. The residue (100 mg) was identical with starting material (87) (TLC).

N,O-Bis[*tert*-butoxycarbonyl]-tyrosyl-thioglycyl-glycine phenyl ester

(93)

To a solution of BOC-Tyr(BOC)-OH (95) (1·25 g, 3·28 mmol)[100] in DCM (20 ml) at -20°C was added DPPCl (70) (816 mg, 3·45 mmol) and NMM (0·4 ml, 3·7 mmol) and the mixture stirred (0·3 h). The hydrobromide (91) (1·0 g, 3 mmol) and additional NMM (0·6 ml, 5·5 mmol) were added. The reaction was allowed to reach room temperature and then stirred overnight (~18 h). After evaporation of solvent the crude residue was washed in the usual way. Drying of the organic phase ($MgSO_4$), filtration and evaporation gave an oily residue which after medium pressure column chromatography (15% EtOAc/DCM, R_f 0·3) afforded the title compound as a white crystalline solid (1·2 g, 62%) which became slightly yellow on standing in air.

mp 76·5-78·5°C

^{1}H n.m.r. (60 MHz) δ 1·35 (9H, s, $(CH_3)_3$ C-), 1·50 (9H, s, $(CH_3)_3$ C-), 3·0 (2H, br m, CH_2 Tyr^1), 4·3 (3H, m, d, 5 Hz, CH Tyr^1, CH_2 Gly^3), 4·5 (2H, d, 5 Hz, CH_2 Glyt^2), 5·3 (1H, d, 7 Hz, NH Tyr^1), 7·0-7·4 (9H, m, aromatic), 7·4 (1H, br m, NH Glyt^2), 9·0 (1H, br t, NH Gly^3).

^{13}C n.m.r. δ 27·7 and 28·3 (q, $(\underline{C}H_3)_3$ C), 37·2 (t, CH_2 Tyr^1), 46·9 (t, CH_2 Glyt^2), 50·1 (t, CH_2 Gly^3), 56·3 (d, CH Tyr^1), 80·6 and 83·5 (s, $(CH_3)_3$ C-), 121·4 (s, C_β, OPh Tyr^1), 126·2-134·0 (4C, C_δ, C_γ, aromatic), 150·1 and 150·4 (s, C_α, OPh Tyr^1), 151·9 (s, C=O, carbonate), 156·0 (s, C=O, carbamate), 166·9 (s, C=O, ester), 172·4 (s, C=O, amide),

200·8 (s, C=S).

I.R. ν_{max}, 3200-3400 (NH), 1750 (C=O, ester, carbonate), 1660 (C=O, amide, carbamate).

U.V. λ_{max}, 264 ($\varepsilon = 1{\cdot}08 \times 10^4$).

Mass (m/e),(FAB +), 588 (M+1, 14), 532 ($M-C_4H_8^+$, 9), 516 ($M-C_4H_8O^+$, 4) 432 (532-BOC, 9).

Elemental Analysis, Found, C, 59·49; H, 6·42; N, 7·06; S, 5·53.

Calc. for $C_{29}H_{37}N_3O_8S$: C, 59·28; H, 6·30; N, 7·16; S, 5·45.

N,O-Bis[*tert*-butoxycarbonyl]-tyrosyl-thioglycyl-glycyl-phenylalanyl-leucine *tert*-butyl ester (94)

A solution of BOC-Tyr(BOC)-Glyt-Gly-OPh (93) (270 mg, 0·46 mmol), H-Phe-Leu-OBu^t (96) (157 mg, 0·47 mmol)[101] and TEA (0·25 ml, ~4 eq.) in THF was refluxed (27 h). The solution was cooled to room temperature and evaporated. The crude oily residue (orange) was subjected to medium pressure column chromatography (25-50% EtOAc/DCM) to give recovered starting material (93) (identical by TLC, 60 MHz ^1H n.m.r.) (60 mg, 0·10 mmol) and the title compound (R_f 0·4, 50% EtOAc/DCM) as a white solid (190 mg, 68% corrected yield).

mp 148-149°C.

^1H n.m.r. δ 0·90 and 0·91 (3H, d, 6 Hz, $(C\underline{H}_3)_2$ CH Leu^5), 1·36, 1·45 and 1·54 (9H, s, Bu^t), 1·5-1·65 (3H, m, $C\underline{H}_2C\underline{H}(CH_3)_2$ Leu^5), 2·95 (2H, m, CH_2 Tyr^1 and CH_2 Phe^4), 3·10 and 3·13 (1H, dd, 13 Hz, 2 Hz, CH_2 Tyr^1 and CH_2 Phe^4), 4·2-4·3 (5H, m, CH_2 Gly^1, Glyt^2, CH Leu^5), 4·46 (1H, 2t, 8·5 Hz, 5·5 Hz, CH Tyr^1), 5·05 (1H, br q, CH Phe^4), 5·38 (1H, d, 7 Hz, NH Tyr^1), 6·81 (1H, d, 8 Hz, NH, Leu^5). 7·05-7·25 (9H, m, aromatic), 7·3 (1H, br t, 4-5 Hz, NH Gly^1), 7·43 (1H, br s, NH Phe^4), 9·15 (1H, t, 4-5 Hz, NH Glyt^2).

^{13}C n.m.r. δ 22·3 and 22·9 (q, CH_3 Leu^5), 25·0 (d, $\underline{C}H(CH_3)_2$ Leu^5)

27·7, 28·1 and 28·4 (q, ($\underline{C}H_3)_3$ C), 37·9, 39·5 and 39·5 (t, $\underline{C}H_2CH(CH_3)_2$ Leu^5, CH_2 Tyr^1, CH_2 Phe^4), 41·9 (t, CH_2 Gly^3), 48·9 (CH_2 Glyt^2), 51·8, 54·2 and 56·0 (d, CH Tyr^1, CH Phe^3, $\underline{C}HCO_2$ CH_3 Leu^5), 80·3, 81·8 and 83·4 (s, $(CH_3)_3$ $\underline{C}$), 121-129·5 (aromatic C), 134·2 and 136·6 (s, C_α Phe , C_γ Tyr^1), 150·2 (s, C_α Tyr^1), 151·8 (s, C=O, carbonate), 156·8 (s, C=O, carbamate), 167·0 (s, C=O, ester), 170·7, 171·8 and 172·0 (s, C=O, amide), 198·5 (s, C=S).

I.R. ν_{max}, 3300 (NH), 1760 (C=O, ester, carbonate), 1690 (C=O, carbamate), 1650 (C=O, amide).

U.V. λ_{max}, 264 (ε = 1·19 x 10^4).

Mass (m/e) (CI), 392 (H-Gly-Phe-Leu-OBu^t, 1·4), 279 (H-Phe-Leu-OH or BOC-Tyr-Glyt$^+$, 48), 260 (279-H_2O, 43).

Elemental Analysis, Found: C, 60·84; H, 7·51; N, 8·19; S, N/D.

Calc. for $C_{42}H_{61}N_5O_{10}S$: C, 60·94; H, 7·38; N, 8·46.

Tyrosyl-thioglycyl-glycyl-phenylalanyl-leucine trifluoroacetate salt (83)

To a solution of BOC-Tyr(BOC)-Glyt-Gly-Phe-Leu-OBu^t (94) (90 mg, 0·11 mmol) in dry DCM (2·7 ml) was added anisole (0·10 ml, excess) and TFA (0·3 ml) and the mixture stirred at room temperature (1·5 h). The solvent was evaporated and ether (10 ml) added to the liquid residue. The slightly coloured (cream) precipitate so formed was separated by decantation of the solvent and then washed with a further ether aliquot (5 ml). The precipitated product (52 mg) consisted of two major components by reverse phase TLC (50:50, MeOH:water) at R_f 0·8 and R_f 0·15. The two were separated and purified by medium pressure column chromatography using reverse phase silica.[113]

The lower R_f component after purification afforded a white powder (25 mg, 31%) identified as the t-butyl ester (99) of the title

compound, primarily by ^{1}H n.m.r. (400 MHz).

^{1}H n.m.r. (400 MHz) δ 0·93 and 0·98 (3H, d, 6·5 Hz, $(C\underline{H}_3)_2$ CH), 1·48 (9H, s, *t*-Bu), 1·62 (2H, m, 9 Hz, 6 Hz, $C\underline{H}_2$ CH Me_2), 1·71 (1H, br m, 6·5 Hz, CH CH_2 $C\underline{H}$ Me_2), 2·98 and 2·99 (1H, dd, 14 Hz, 10 Hz and 14 Hz, 9 Hz, CH_2 Tyr^1 and CH_2 Phe^4), 3·21 and 3·24 (1H, dd, 14 Hz, 4·5 Hz and 14 Hz, 6 Hz, CH_2 Tyr^1 and CH_2 Phe^4), 4·13 (1H, dd, 9 Hz, 6 Hz, CH), 4·18, 4·23, 4·32 and 4·36 (1H, d, 17 Hz, CH_2 Glyt^2 and CH_2 Gly^1), 4·34 (1H, dd, 9 Hz, 6 Hz, CH), 4·74 (1H, dd, 10 Hz, 4·5 Hz, CH Tyr^1 or CH Phe^4), 6·85 and 7·15 (2H, d, 8·5 Hz, Tyr^1, aromatic), 7·2 -7·3 (5H, m, aromatic). (Other protons exchanged with solvent, D_2O/MeOH-d_4.)
Mass (m/e) (CI), 392 (Gly-Phe-Leu-OBu^t, 9), 336 (392-Bu^t, 75), 279 (H-Phe-Leu-OH, 96), 261 (279-H_2O, 100).

After a similar purification the higher R_f component (83) (15 mg, 20%) was obtained as a white powder and identified as the title compound.

^{1}H n.m.r. (400 MHz) δ 0·95 and 0·98 (3H, d, 6 Hz, $(C\underline{H}_3)_2$ CH), 1·65 (2H, br t, 7 Hz, $C\underline{H}_2$ CH Me_2), 1·72 (1H, br m, 6 Hz, CH_2 $C\underline{H}$ Me_2), 2·98 and 3·01 (1H, dd, 14 Hz, 9 Hz and 14 Hz, 10 Hz, CH_2 Tyr^1 and CH_2 Phe^4), 3·21 and 3·24 (1H, dd, 14 Hz, 6 Hz and 14 Hz, 4·5 Hz, CH_2 Tyr^1 and CH_2 Phe^4), 4·11, 4·18, 4·48 and 4·58 (1H, d, 16 Hz, CH_2 Glyt^2 and CH_2 Gly^3), 4·15 (1H, dd, 9 Hz, 6 Hz, CH Tyr^1 or CH Phe^4), 4·35 (1H, t, 7 Hz, CH Leu^5), 4·71 (1H, dd, 10 Hz, 4·5 Hz, CH Tyr^1 or CH Phe^4), 6·85 and 7·17 (2H, d, 8·5 Hz, Tyr^1, aromatic), 7·25 (5H, m, aromatic). (Other protons exchanged with solvent, D_2O/MeOH-d_4).
Mass (m/e) (FAB+), 572 (M+1, 5·5), 556 (M-NH_2, 1), 538 (556-H_2O, 1), 336 (Gly-Phe-Leu-OH, 2·5), 279 (H-Phe-Leu-OH, 23), 136 ($\overset{+}{H_2}N$ = $CHCH_2C_6H_4OH$, 64), 120 ($\overset{+}{H_2}N$ = CH CH_2 Ph, 100), 91 ($C_7H_7^+$, 23).

Elemental Analysis, Found: C, 51·11; H, 6·02; N, 9·87. Calc. for $C_{30}H_{38}N_5O_8SF_3H_2O$: C, 51·21; H, 5·69; N, 9·95.

The t-butyl ester (99) could be converted to the title compound (83) (50%) under identical conditions except that the reaction was stirred for a longer duration (6 h).

N-Triphenylmethyl-2-aziridine carboxylic acid methyl ester (139)

The title compound[68,69] was prepared in a three step sequence from serine methyl ester hydrochloride salt (136) following the procedure[68,69] described for the corresponding 3-methyl derivative (140). Crystallization from petroleum ether (60-80) afforded colourless crystals.

mp 106·5 - 108°C

^{1}H n.m.r. δ 1·4 (1H, dd, 1·5 Hz, 6 Hz, βH trans), 1·9 (1H, dd, 2·5 Hz, 6 Hz, αH), 2·3 (1H, dd, 1·5 Hz, 2·5 Hz, βH cis), 3·75 (3H, s, OCH_3), 7·2-7·6 (15H, m, aromatic).

^{13}C n.m.r. δ 28·6 (t, $\underline{C}H_2N$), 31·7 (d, $N\underline{C}HCO_2$ CH_3), 51·9 (q, OCH_3), 74·4 (s, Ph_3 $\underline{C}$-N), 126·9-129·3 (3C, aromatic), 143·6 (s, Cα aromatic), 171·8 (s, C=O, ester).

I.R. ν_{max}, 1750 (C=O, ester)

Mass (m/e) (CI), 285 (M-CO_2 CH_3, 7), 243 (Ph_3C^+, 100)

Elemental Analysis, Found: C, 80·67; H, 6·21; N, 3·81. Calc. for $C_{23}H_{21}NO_2$: C, 80·47; H, 6·12; N, 4·08.

N-[N'-*tert*-Butoxycarbonylvalyl]-2-aziridine carboxylic acid methyl ester (142)

To a solution of Tr-Azy-OMe (139) (1·0 g, 2·9 mmol) in $CHCl_3$ (4 ml):MeOH (4 ml) was added TFA (4 ml) slowly (0·1 h) dropwise. The reaction mixture was stirred at room temperature (3 h). The

slightly coloured (yellow) solution was evaporated to remove solvent and then dry ether (5 ml) added and the solution re-evaporated. This process was repeated twice more to remove all traces of TFA. The crude residue was blown dry (N_2) overnight and used as follows.

To a cooled (-20°C) solution of BOC-Val-OH (141) (696 mg, 3·2 mmol) in DCM (20 ml) was added DPPCl (70) (758 mg, 3·2 mmol) and NMM (0·35 ml, 3·2 mmol) and the mixture stirred (0·3 h). A solution of the crude aziridine in DCM (5 ml) neutralized with NMM (0·35 ml, 3·2 mmol) was then added. The solution was allowed to warm up to room temperature overnight (16 h). The reaction mixture was evaporated and taken into EtOAc (50 ml) and washed in the normal way. After drying ($MgSO_4$) and evaporation of the organic phase the crude residue was subjected to medium pressure column chromatography (5% EtOAc/DCM). The fractions containing the desired product were combined and evaporated giving the title compound as a white solid (340 mg, 39%) crystallized from DCM/petroleum ether (40-60) as colourless needles.

mp 56·5 - 58·0°C

^{1}H n.m.r. δ 1·0 (6H, t, 7 Hz, $(C\underline{H}_3)_2$ CH), 1·4 (9H, s, $(C\underline{H}_3)_3$ C), 2·2 (1H, m, C<u>H</u> Me_2), 2·7 (2H, m, CH_2 N), 3·25 (1H, dd, 3 Hz, 5·5 Hz, NC<u>H</u>), 3·8 (3H, s, OCH_3), 4·2 (1H, dd, 9 Hz, 4·5 Hz, NH C<u>H</u>), 5·2 (1H, br d, 9 Hz, NH).

^{13}C n.m.r. δ 17·4 and 19·3 (q, <u>Me</u>$_2$ CH), 28·3 (q, <u>Me</u>$_3$ C), 30·6 (t, CH_2 N), 31·6 (d, C<u>H</u> Me_2), 34·4 (d, C<u>H</u> N), 52·3 (q, OMe), 60·6 (d, NH C<u>H</u>), 79·7 (s, Me_3 C), 155·7 (s, C=O, carbamate), 168·6 (s, C=O, ester), 182·8 (s, C=O, amide).

I.R. ν_{max}, 3340 (NH), 1750 (C=O, ester), 1705 (C=O, carbamate), 1680 (C=O, amide).

Mass (m/e) (CI), 301 (M+1, 0·5), 245 ($M-C_4H_8^+$, 3·5), 227 ($M-C_4H_8CO^+$, 5), 200 (M-BOC or M-(Azy-OMe), 6), 116 ($Bu^tO-CO-NH^+$, 40),

102 (Bu^tO-CO^+, 100).

Elemental Analysis, Found: C, 56·02; H, 8·08; N, 9·45. Calc. for $C_{14}H_{24}N_2O_5$: C, 56·00; H, 8·00; N, 9·33.

Also isolated from the reaction mixture by column chromatography was a white solid (90 mg, 9%) identified as N-tert-butoxycarbonylvalyl-β-chloroalanine (145) from the following data.

^{1}H n.m.r. δ 1·0 (6H, t (2d), 6 Hz, $(CH_3)_2$ CH), 1·4 (9H, s, $(CH_3)_3$ C), 2·1 (1H, m, CH Me_2), 3·8 (3H, s, OCH_3), 3·9 (2H, t (dd), 3·5 Hz, CH_2 Cl), 4·05 (1H, m, CH CH_2 Cl), 5·0 (1H, m, CH Val^1), 5·3 (1H, br d, 9 Hz, NH Val^1), 7·15 (1H, d, 8 Hz, NH).

^{13}C n.m.r. δ 17·6 and 19·2 (q, Me_2 CH, 28·3 (q, Me_3 C), 30·9 (d, CH Me_2), 44·7 (t, CH_2 Cl), 52·9 (q, OMe), 53·3 (d, CH CH_2 Cl), 59·6 (d, CH NH), 80·1 (s, Me_3 C), 155·8 (s, C=O, carbamate), 169·1 (s, C=O, ester), 171·9 (s, C=O, amide).

Mass (m/e) (CI), 337 (M^+, 1), 309 (M-MeOH, 2), 281 (M-$C_4H_8^+$, 9), 237 (M-BOC, 42), 72 ($C_4H_8O^+$, 100), 57 ($C_4H_9^+$, 73).

Reaction of N-[N'-*tert*-butoxycarbonylvalyl]-2-aziridine carboxylic acid methyl ester (142) with Lawesson's reagent (25)

A suspension of BOC-Val-Azy-OMe (142) (50 mg, 0·17 mmol) and Lawesson's reagent (25) (37 mg, 0·09 mmol) in dry xylene (5 ml) was heated at 70-80°C (0·1 h). The resultant solution was cooled to room temperature (0·5 h) and the solvent evaporated to give a slightly coloured (yellow) oily product. The latter was subjected to medium pressure column chromatography (4% EtOAc/DCM). Fractions containing the desired product (R_f 0·4) were combined and evaporated to afford a slightly coloured oil (10 mg, 18·5%). This product was not stable and decomposed in air (2-3 d).

^{1}H n.m.r. (60 MHz) δ 1·0 and 1·05 (3H, d, 7 Hz, CH (CH_3)$_2$), 1·4 (9H, s, *t*-Bu), 2·2 (~1H, m, C$\underline{H}$ Me_2), 2·9 (2H, m, aziridine βH), 3·35 (1H, dd, 3 Hz, 6 Hz, aziridine αH), 3·7 (3H, s, OCH_3), 4·2 (1H, m, NH C$\underline{H}$), 5·2 (1H, br d, NH).

Other products were not observed in this reaction although a small amount of a component identified as unreacted BOC-Val-Azy-OMe (142) (~5 mg, 10%) was also isolated from column chromatography.

Reaction of N-[N'-*tert*-butoxycarbonylvalyl]-2-aziridine carboxylic acid methyl ester (142) with "Belleau's reagent" (27a)

A solution of BOC-Val-Azy-OMe (142) (155 mg, 0·52 mmol) in THF (5 ml) was treated with "Belleau's reagent" (27a) (150 mg, 0·28 mmol). The mixture was stirred at room temperature until none of the starting dipeptide (142) remained, as monitored by TLC (35% EtOAc/DCM), and the solvent evaporated. The residue was subjected to medium pressure column chromatography (40% EtOAc/petroleum ether 40-60) and the fractions containing the desired product (R_f 0·5) combined and evaporated. This afforded a colourless oil which, after rigorous solvent removal, solidified to a white solid (120 mg).

^{1}H n.m.r. (60 MHz). This spectrum was complex and could not be fully assigned but contained the following signals: 0·9 (6H, m, (CH_3)$_2$ CH), 1·4 (9H, s, Bu^t), 2·1 (2H, m), 3·7 (3H, d, OMe), 7-8 (9-10H, m, aromatic).

Mass (m/e) 464 (0·4), 448 (0·4), 331 (2·6), 278 (3), 105 (100), 57 (C_4H_9, 89).

Reaction of BOC-Val-Azy-OMe (142) with Phosphorus pentasulphide

A solution of BOC-Val-Azy-OMe (142) (400 mg, 1·33 mmol) in THF (10 ml) was treated with phosphorus pentasulphide (P_2S_5) (327 mg, 1·47 mmol). The suspension was subjected to ultrasonic vibration, using an ultrasonic cleaning bath, at 30°C (3·7 h). The mixture was evaporated and the residue partitioned between EtOAc (50 ml) and aqueous citric acid (5%, 25 ml). The heterogeneous mixture was filtered to remove an insoluble white solid. The filtrate was separated and the organic layer washed with water (3 x 20 ml) before drying ($MgSO_4$), filtration and evaporation. The slightly coloured (yellow) oily residue was subjected to medium pressure column chromatography (40% EtOAc/DCM) and the first few fractions, containing a mixture of components combined and evaporated to afford a colourless oil (150 mg).

^{1}H n.m.r. (60 MHz) δ 0·95 and 1·0 (3H, d, 7 Hz, Me_2 CH), 1·4 (9H, s, *t*-Bu), 3·6 (3H, br s, OCH_3) (Other signals comprised several small multiplets (br) between 3 and 5 ppm and were unresolved.)
Mass (m/e) (CI), 335 (11), 317 (37·5), 279 (100), 261 (88·4), 235 (61), 217 (24).

N-tert-Butoxycarbonyl-valine piperidide (132a)

To a cooled (-20°C) solution of BOC-Val-OH (141) (2·17 g, 10 mmol) in dry DCM (30 ml) was added DPPCl (70) (2 ml, 10·5 mmol) and NMM (1·1 ml, 10·1 mmol) and the mixture stirred (0·3 h). Piperidine (2 ml, 20 mmol) was added slowly (0·2 h) and the reaction mixture allowed to warm to room temperature (2 h) and stirred for a further period (5 h). The solvent was evaporated and the crude residue taken into EtOAc and washed in the normal way. After drying ($MgSO_4$) the organic phase was evaporated and subjected to medium pressure column

chromatography (10% EtOAc/DCM). The fractions containing the desired product (R_f 0·3) were combined and evaporated giving the title compound as a colourless oil. After several evaporations from ether the oily product solidified in air (7 d) to a colourless semi-crystalline solid (2·7 g, 95%).

mp 53-54·5°C

^{1}H n.m.r. δ 0·9 and 0·95 (3H, d, 8 Hz, $(C\underline{H}_3)_2$ CH), 1·40 (9H, s, $(C\underline{H}_3)_3$ C), 1·60 (6H, br s, CH_2 piperidine), 1·9 (1H, m, C<u>H</u> $(CH_3)_2$), 3·5 (4H, br m, CH_2 N), 4·5 (1H, dd, 7 Hz, 8 Hz, C<u>H</u> NH), 5·45 (1H, d, 8 Hz, NH).

^{13}C n.m.r. δ 17·1 and 19·7 (q, $(\underline{C}H_3)_2$ CH), 24·6. 25·7 and 26·6 (t, CH_2), 28·4 (q, $(\underline{C}H_3)_3$ C), 31·6 (d, <u>C</u>H $(CH_3)_2$), 43·1 and 46·8 (t, CH_2 N), 54·7 (d, <u>C</u>H NH), 79·1 (s, <u>C</u> $(CH_3)_3$), 155·9 (s, C=O, carbamate), 170·2 (s, C=O, amide).

I.R. ν_{max}, 3300 (NH), 1705 (C=O, carbamate), 1625 (C=O, amide).

Mass (m/e) (CI), 285 (M+1, 42), 229 (M-C_4H_8, 100), 185 (M-BOC, 79), 72 ($C_4H_8O^+$, 36).

Elemental Analysis, Found: C, 63·59; H, 10·10; N, 10·12. Calc. for $C_{15}H_{28}N_2O_3$: C, 63·38; H, 9·86; N, 9·86.

N-tert-Butoxycarbonyl-valine thiopiperidide (132b)

A suspension of BOC-Val-Piperidide (132a) (827 mg, 2·9 mmol) and Lawesson's reagent (25) (655 mg, 1·6 mmol) in toluene (15 ml) was heated at 80°C (4·5 h). The resultant solution was cooled to room temperature and evaporated. The crude residue was subejcted to medium pressure column chromatography (DCM) and the fractions containing the desired product (132b) (R_f 0·25) combined and evaporated. This gave the title compound as a colourless oil (210 mg, 24%). Some of the starting piperidide (132a) was also recovered (150 mg, 0·5 mmol)

by elution with a more polar solvent (5% EtOAc/DCM).

^{1}H n.m.r. δ 0·95 (6H, t, 8 Hz, CH $\underline{Me}_2$), 1·4 (9H, s, *t*-Bu), 1·75 (6H, br s, CH_2 of piperidine), 2·05 (1H, m, $C\underline{H}$ Me_2), 3·9 (2H, br s, CH_2 N), 4·3 (2H, br m, CH_2 N), 4·6 (1H, dd, 8 Hz, 9·5 Hz, $C\underline{H}$ NH), 5·7 (1H, br d, 9·5 Hz, NH).

^{13}C n.m.r. δ 17·2 and 19·9 (q, $\underline{Me}_2$ CH), 24·4, 25·5 and 27·0 (t, CH_2), 28·4 (q, $\underline{Me}_3$ C), 34·3 (d, $C\underline{H}$ Me_2), 51·3 and 52·1 (t, CH_2 N), 59·2 (d, $C\underline{H}$ NH), 79·2 (s, Me_3 $\underline{C}$), 155·5 (s, C=O carbamate), 202·7 (s, C=S).

Attempted S-methylation of N-tert-butoxycarbonyl-valine thiopiperidide (132b)[42,43]

A solution of BOC-Valt-Pip (132b) (230 mg, 0·77 mmol) in dry THF (10 ml) was treated with methyl iodide (0·25 ml, 4 mmol) and the mixture stirred at room temperature overnight (16 h) in the dark. The reaction mixture was evaporated to afford a slightly coloured (yellow) oil. The ^{1}H n.m.r. (60 MHz) of this material was identical in all respects to starting piperidide.

The oil was re-dissolved in dry THF (13 ml) and the solution treated with methyl iodide (0·5 ml, 8 mmol) and refluxed (48 h). After evaporation a coloured (yellow) oil was again obtained. ^{1}H n.m.r. (60 MHz) of this material was identical with starting material (132b).

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APPENDIX

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ENDOTHIOPEPTIDES

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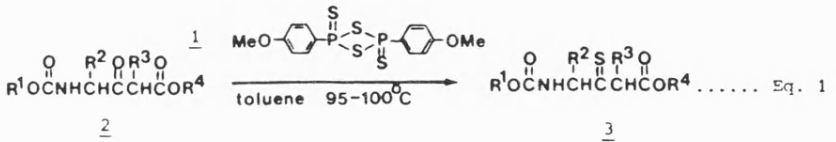
Abstract - N-protected dipeptide esters have been converted into the corresponding protected endothiopeptides[1†] using Lawesson's reagent,[2] 1. Methods of amino and carboxyl deprotection, and coupling of the thiopeptides with amino acids have been defined and used to prepare two examples of novel N-protected endothiotripeptide esters with a single thioamide link. Possible effects of the thioamide bond on the conformation of the dipeptide esters are considered.

Until recently there were very few reports in the literature concerning the synthesis of endothiopeptides.[1,3] The introduction of Lawesson's reagent by which amides are readily converted in high yields into thioamides[2] has given new impetus[3a,b] to this synthetic objective. We are prompted by these recent disclosures[3] to report our interest in sulphur analogues of biologically important peptides. Our complementary studies in this area led us to examine the scope of this reagent and to determine whether the existing protection, deprotection, and coupling procedures used in conventional peptide synthesis are generally applicable to these new systems.

It has been shown that both the amino and carboxyl functions react with Lawesson's reagent[2] 1 and so N-protected dipeptide esters, 2 were used. The thiopeptides, 3 shown in Table 1 were readily prepared from the corresponding peptides, 2 in high yields according to the generalised equation 1.

The same reaction conditions have been employed for each substrate but no difference in rate of thionation of the different dipeptide amide funtions has been detected, thus it is improbable that selective thionation of tri- and higher peptides will easily be achieved. High resolution mass measurements (Table 1) correspond to the expected molecular formulae; the fragmentation patterns conform to those previously described.[3a] All compounds were homogeneous by TLC and NMR.

The ^{13}C NMR, ^{1}H NMR and UV data on these compounds are presented in Table 2 and the linear relationship described by Clausen et al[3a] whereby $\delta(C{=}S)=1.62$. $\delta(C{=}O)-74.15$ used to obtain the calculated ^{13}C chemical shift values quoted. All data are consistent with N-Z-endiothiodipeptide esters, 3.



2 3 ..... Eq. 1

† The name endothiopeptide has been used previously for peptides with the thioamide link in the backbone.[1,2]

The protective benzyloxycarbonyl (Z) group was removed from the doubly-protected endothiopeptides, 3 by hydrogen bromide in acetic acid at room temperature[4] and the tert-butoxycarbonyl (Boc) group similarly using trifluoroacetic acid.[5] In each case very good yields of N-deprotected compounds were obtained in the former as the hydrobromide and in the latter as the trifluoroacetate salt.

The NMR spectral date of the N-deprotect- compounds, 4 are collected in Table 3. Both 4a and 4b have prominent ions in their mass spectra at M-81 due to their parent amines. Fragmentation in 4a occurs via the loss of methanol and then CO and analogously in 4b where the tropylium ion also figures prominently. In each case the base peak is at 30 mass units $[CH_2{=}NH_2]^+$.

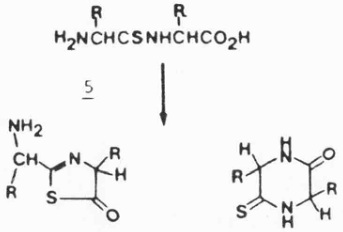
Attempts to remove the carboxyl protecting groups from the doubly-protected peptide, 3a, selectively using alkaline hydrolysis methods[3b] result in deep orange/red coloured mixtures (by TLC). Only poor yields of the free acid were obtained and so this deprotection method was not pursued.

Removal of both amino and carboxyl protecting groups of 3a, 3b and 3d by HBr/AcOH at 60° gave the endothiopeptides, 5, representing for the first time the preparation of totally deprotected systems.

Surprisingly, double deprotection of the ester 3d takes place much more rapidly than 3a in the presence of hydrogen bromide in acetic acid.

The ^1H- and ^{13}C- NMR spectra of 5 are consistent with those expected for the endothiodipeptides (see Table 3).

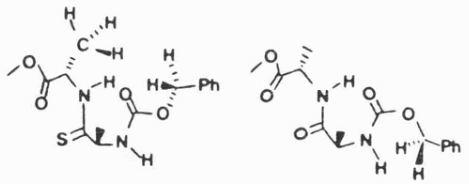
In the case of 5d and 3d, the main difference in the ^{13}C-NMR spectrum is the absence of resonance at about 52.0 ppm for 5d, the characteristic position of the methoxyl carbon. Spectroscopic data were in accord with endothioaminocarboxylic acids. The mass spectra of these compounds give highest mass ions of 100 mass units ($-HBr-H_2O$) lower than that expected and may be due to ions corresponding to thiazolin-5-one, 7, or thioketopiperazine structures, 8.



5 → 7 Eq. 2 8

In an initial study of the conformational consequences of the introduction of the thioamide group into petides, differential nOe spectra were taken on compounds 2e and 3e. Each spectrum was consistent with favoured conformations 9 and 10 resulting from hydrogen bonding between the amide N-H and carbamate carbonyl oxygen atom. In the case of the thioamide 3e a strong (33%) enhancement of the methylene protons was observed when the Ala-methyl group was continuously irradiated. There was no corresponding enhancement in 2e, but instead an enhancement (14%) of the signal due to the carbamate N-H proton when the methylene protons were irradiated. These observations may correspond to different preferred conformations about the carbamate C-O bond.

The thioamide proton is more acidic than the amide one and this could bring about stronger hydrogen bonding in 3e. The resulting



10 9

depletion in electron density in the carbamate C=O might result in more double bond character in its C-O bond favouring the conformation about the carbamate bond shown in 3e. In conclusion, difference nOe at 400 MHz indicates a significantly populated conformation (10) for the endothiopeptide 3e.

It has been reported that thionation of peptides with Lawesson's reagent takes place with no appreciable degree of racemization. This claim is based on a comparison of optical rotations of samples of N-protected dipeptide

Table 1: Yields and Physical Data for N-protected Endothiodipeptide esters, 3.

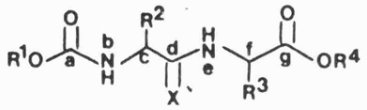
Substituent					Product (3)[a]	Yield	mp	Mol.formula	Accurate mass	
	R^1	R^2	R^3	R^4		%	°C		Calc.	Obs.
3a	$PhCH_2$	H	H	CH_3	Z-Glyt-Gly-OMe	92	89.0- 90.0	$C_{13}H_{16}N_2O_4S$	296.0827	296.0833
b	$(CH_3)_3C$	H	H	CH_2Ph	BOC-Glyt-Gly-OBzl	89	103.5-105.0	$C_{16}H_{22}N_2O_4S$	338.1295	338.1302
c	$PhCH_2$	$PhCH_2$	H	CH_3	Z-Phet-Gly- OMe	83	68.0- 69.5	$C_{20}H_{22}N_2O_4S$	386.1295	338.1301
d	$PhCH_2$	$CH_3CH_2CH(CH_3)$	H	CH_3	Z-iLet-Gly-OMe	86	-[b]	$C_{17}H_{24}N_2O_4S$	352.1451	352.1459
e	$PhCH_2$	CH_3	CH_3	CH_3	Z-Alat-Ala-OMe	100	-[b]	$C_{15}H_{20}N_2O_4S$	324.1139	324.1151
f	$PhCH_2$	H	H	Ph	Z-Glyt-Gly-OPh	70	92.5- 94.0	$C_{18}H_{18}N_2O_4S$	264.0557[c]	264.0557

a The symbols for the thiocarbonyl derivatives are those used by du Vigneaud et al[8].

b Products obtained as colourless gums.

c M-PhOH, molecular ion not observed.

Table 2: ^{13}C-NMR, ^{1}H-NMR[a] and UV Data for N-Z-dipeptide esters, 2 and N-Z-endothiodipeptide esters, 3.

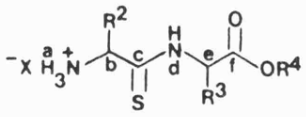


2 : X = O
3 : X = S

COMPOUND		^{13}C-NMR Chemical shift (ppm)					^{1}H-NMR Chemical shift (ppm)				UV ($CHCl_3$) λmax (ε)(nm)	
		a	c	d(calc.)	f	g	b	c	e	f		
2a	Z-Gly-Gly-OMe	156.7	44.5	169.7(-)	41.2	170.3	5.90	3.90	7.00	4.00	-	-
b	BOC-Gly-Gly-OBzl	156.2	44.3	169.7(-)	41.3	170.2	5.65	3.95	7.15	4.05	-	-
c	Z-Phe-Gly-OMe	156.0	56.3	171.3(-)	41.2	169.8	5.50	4.50	6.60	3.95	-	-
d	Z-iLe-Gly-OMe	156.5	59.8	172.0(-)	41.1	170.2	5.80	4.20	7.10	4.00	-	-
e	Z-Ala-Ala-OMe	155.8	50.8	173.2(-)	48.3	172.3	5.60	4.40	6.90	4.40	-	-
f	Z-Gly-Gly-OPh[b]	156.7	43.8	168.4(-)	41.1	170.1	8.15	3.80	7.00	4.20	-	-
3a	Z-Glyt-Gly-OMe	156.8	52.4	200.5(200.8)	46.7	168.8	5.90	4.25	8.60	4.40	266	(12,700)
b	Z-Glyt-Gly-OBzl	156.3	52.1	201.0(200.8)	46.5	168.4	5.45	4.20	8.70	4.40	266	(12,800)
c	Z-Phet-Gly-OMe	155.9	62.6	204.1(203.4)	46.8	168.5	5.80	4.80	8.30	4.20	269	(12,300)
d	Z-iLet-Gly-OMe	156.5	65.3	205.8(204.5)	46.6	168.7	5.95	4.45	9.20	4.30	269	(11,300)
e	Z-Alat-Ala-OMe	155.9	56.3	205.5(206.4)	53.4	172.2	5.90	5.10	8.70	4.70	270	(10,900)
f	Z-Glyt-Gly-OPh	156.8	52.0	201.0(198.7)	47.0	167.2	5.80	4.20	8.70	4.55	265	(16,060)

a The spectra were determined in $CDCl_3$ at 30°C unless otherwise stated.
b The spectra of 2f were obtained in $CDCl_3$: DMSO-d_6, 1:1 at 60°.

Table 3: Spectral and Physical Data for Endothiodipeptide esters, 4 and Endthiodipeptides, 5.

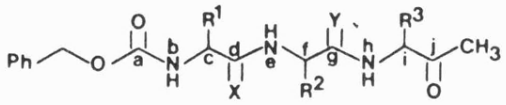


4: R^2, R^3, R^4 as for compounds: 2 and 3
5: R^2, R^3 as for compounds: 2 and 3, R^4=H
4a, 5a, 5d: X=Br
4b: X=CF_3CO_2

Compound	^{1}H-NMR					^{13}C-NMR					mp
	Solvent	Chemical Shift (ppm)				Chemical Shift (ppm)					°C
		a	b	d	e	b	c	e	f	g[a]	
4a	DMSO-d^6	8.25	3.95	11.00	4.40	42.7	198.0	47.8	169.5	-	171-173(d)
4b	DMSO-d^6	8.80	3.95	-	4.50	45.8	196.8	46.5	167.5	66.1	129-131(d)
5a	D_2O	-	3.90	-	4.10					-	91- 94
5d	D_2O	-	4.05	-	4.50	63.5	200.8	47.6	172.1	-	175-179(d)

a where R =$PhCH_2$, g is shift for methylene carbon.

Table 4: Spectral and Physical Properties of N-Z-endothiotripeptide esters, 6.



6a : X = O, Y = S
6b : X = S, Y = O

	Substituent			Yield	mp	Chemical shift (ppm) [c] ^{1}H-NMR						^{13}C-NMR						
	R^1	R^2	R^3	%	°C	b	c	e	f	h	i	a	c	d	f	g	j	i
6a	CH_3	H	H	79	108.0-109.0	5.9	4.3	7.55	4.3	9.00	4.3	156.5	51.2	173.6	50.1	200.3	46.9	169.0
6b	H	H	H	47	130.5-132.5	7.3	4.2	9.6	4.3	8.25	3.9	156.7	48.1	200.2	40.9	169.9	52.0	167.8

a Acc. mass = 367.1215, calc. for $C_{16}H_{21}N_3O_5S$ = 367.1197

b Acc. mass = 353.1044, calc. for $C_{15}H_{19}N_3O_5S$ = 353.1041

c The spectra were determined in $CDCl_3$.

esters, obtained by dethiation of the corresponding endothiopeptides prepared using the reagent, with samples of original starting N-protected dipeptide esters. Recent studies[10] have shown that N-alkyloxycarbonyl protected peptides are much more resistant to racemization than N-acyl protected peptides under reaction conditions appropriate for new peptide bond formation. As a consequence of the above we expected our peptides to retain their stereo-chemistry and used high resolution (400 MHz) ^{1}H-NMR spectroscopy to confirm this expectation as follows. The methyl ester 3e, Z-Alat-Ala-OMe contains two chiral centres and so racemization at either centre would give rise to a mixture of diastereoisomers. The ^{1}H-NMR spectrum shows overlapping doublets (1.46 and 1.47, 3H, J=7Hz) due to the methyl groups and two pentets (4.60 and 5.05, 1H, J=7Hz) due to the two methine protons further coupled (J=7Hz) to N-H. Decoupling from N-H reduced each pentet to a quartet. The ^{13}C-NMR spectrum (Table 2) contains thirteen lines only. The foregoing data can only be explained in terms of one diastereoisomer. The high signal to noise ratio and the sharpness of the signals in the ^{1}H-NMR spectrum would allow the detection of isomeric impurities down to perhaps 2-3%; only signals due to 3e were present in its NMR spectra.

The feasibility of synthesising higher endothiopeptides by coupling with N-protected amino acids at the terminal amino group was confirmed by the reaction of the hydrobromide salt of the endothiopeptide ester 4a with Z-Ala-OH to give Z-Ala-Glyt-Gly-OMe 6a. This preparation was carried out using standard conditions[6] whereby the salt was neutralised with triethylamine and the coupling promoted by dicyclohexylcarbodiimide (DDC). This is represented in Equation 3.

Z-Ala-OH + HBr.Glyt-Gly-OMe 4a $\xrightarrow{\text{DCC, 20hr.}}$ Z-Ala-Glyt-Gly-OMe 6a .... Eq. 3

Having branched from the amino terminal, we investigated the alternative coupling at the C-terminal end of an N-protected endothiopeptide ester. This was achieved by utilizing a phenyl ester protecting group (first proposed by Galpin et al[7]). Thus Z-Glyt-Gly-Gly-OMe, 6b, has been prepared from Z-Glyt-Gly-OPh, 3f, and glycine methyl ester according to Equation 4.

Z-Glyt-Gly-OPh 3f + HCl.Gly-OMe $\xrightarrow[\text{reflux 8hr.}]{\text{TEA/IPA}}$ Z-Glyt-Gly-Gly-OMe 6b ... Eq. 4

Attempts to improve the relatively poor yield (47%) using an ester group which was more susceptible to nucleophilic displacement, eg p-nitrophenyl, by a free amino group have been unsuccessful. Thionation of Z-Gly-Gly-OnPh with 1 afforded several inseparable, unidentified products. Significantly p-nitrophenol was also obtained from the reaction so that thionation possibly led to an intramolecular cyclization whereby the nitrophenate anion was displaced by the thiono group.

To sum up, we have extended the range of doubly protected dipeptide substrates for which thionation using Lawesson's reagent proceeds in high yield, defined a major conformer of an N-Z-endothiopeptide ester and shown that the conventional peptide deprotection and coupling procedures are applicable to the synthesis of endothiopeptides from smaller units containing the thioamide bond.

EXPERIMENTAL

The abbreviations used are those in common use and all the amino-acids have the L-configuration. Other abbreviations are as follows* TEA. triethylamine; DCM, dichloromethane; DCC, N,N'-dicyclohexylcarbodiimide; nPh, 4-nitrophenyl; DCU, N,N'-dicyclohexylurea; IPA, isopropanol. Silica gel 60 (Merck No: 7747) was used. The ^{1}H-NMR spectra were determined at 100 MHz using a Jeol PS100 spectrometer, unless otherwise stated, and ^{13}C-NMR spectra at 22.5 MHz using a Jeol FX90Q spectrometer. The ^{1}H-NMR spectra of 2e and 3e were determined on A Brucker WH400 with a pulse length of 4μs at an angle of 45-50°. The differential nOe spectra were run at 310 K with GM (LB=0.85) with 40 scans accumulated for each spectrum. Tetramethylsilane was used as internal standard. Mass spectra were recorded by PCMU (Harwell) at 70eV. Lawesson's reagent, 1, was either prepared by the literature procedure[2] or a commercial sample (Aldrich Ltd) was used.

Z-Ala-Ala-OMe, 2e, was prepared from the free acid by a standard esterification method[9] ($SOCl_2$/MeOH). Z-Gly-Gly-OPh, 2f, was prepared using the method of Galpin et al[7], from the free acid (Z-Gly-Gly-OH).

Preparation of N-protected endothiopeptide esters, 3.

Z-Phet-Gly-OMe, 3c, Z-Phe-Gly-OMe, 2c (460 mg, 1.24 mM) and Lawesson's reagent[2], 1 (250 mg, 0.62 mM) were heated together at 95-100° in sodium dried toluene (20 cm^3) for 2 hr. The reaction was followed by TLC on silica gel (10%, ethyl acetate-dichloromethane). Evaporation in vacuo gave a pale yellow oil which was chromatographed (10% EtOAc-DCM, flash chromatography) giving 3c (400 mg, 1.04 mM, 84%), mpt: 68-69.5°C as a white solid which could be recrystallized from MeOH/H_2O. 3d And 3e were obtained as colourless oils.

Removal of the Z-group from the N-Z-endothiopeptide esters 3.

$Br^-H^+_2$-Glyt-Gly-OMe, 4a. 3a,(640 mg. 2.2 mM) was stirred for 1 hour at 20°C in HBr/AcOH (48% w/v, 10 cm^3) in a flask fitted with a $CaCl_2$ drying tube. Dry ether (50 cm^3) was then added to precipitate more white solid and the whole then filtered and the residue washed with ether. This gave 4a (500 mg, 2.1 mM, 95% yield) as a white solid, mpt: 171-173°C (decomp). (Found: $M^{+\cdot}$-HBr, 162.0475. $C_5H_{10}N_2O_2S$ requires 162.0461).

Simultaneous removal of the amino and carboxyl-protective groups.

$Br^-H_2^+$-Glyt-Gly-OH, 5a. Z-Glyt-Gly-OMe 3a (190 mg, 0.64 mM) and HBr/AcOH (48% w/v, 10 cm^3) were stirred at 55°C in a flask fitted with a $CaCl_2$ drying tube and a reflux condenser for 6 hr. (1.5 hr. for 3d). On cooling, ether (50 cm^3) was added to precipitate a white solid* which was filtered and washed with a small amount of ether. This gave 5a (140 mg, 0.61 mM, 95% yield) as a slightly coloured solid, mpt: 91-94°C. (Found: M^+-HBr-H_2O, 130.0201. $C_4H_6N_2O_2S$ requires 130.0200).

*For the deprotection of Z-Ilet-Gly-OMe, 3d, a reaction time of 1.5 hr at 20°C was sufficient. Addition of ether did not precipitate the hydrobromide 5d but the ether layer was extracted with H_2O (2 x 50 cm^3) and the aqueous extract evaporated under vacuum to give a pale orange solid (75 mg, 0.25 mM, 83% yield), mpt: 175-179°C (decomp.)

Removal of the Boc-group from 3.

$TFA^-H_2^+$-Glyt-Gly-OBzl, 4b, Boc-Glyt-Gly-OBzl, 3a (169 mg, 0.5 mM) was stirred in a solution of 90% TFA (10 cm^3) at RT for 0.5 hr. The excess TFA was removed by toluene azeotrope (2 x 20 cm^3) in vacuo to give a viscous oil which solidified on standing to an off-white solid, 4b, (140 mg, 0.47 mM, 94% yield), mpt: 129-131°C (decomp). (Found: M^+-TFA, 238.0769 $C_{11}H_{14}N_2O_2S$ requires 238.0773).

Preparation of N-Z-endothiotripeptide esters, 6

Z-Ala-Glyt-Gly-OMe, 6a. A suspension of the salt, 4a (242 mg, 1.0 mM) and N-Benzyloxycarbonylalanine (223 mg, 1.0 mM) in DCM (5 cm^3) was added over 5 min. and the suspension stirred for a further 5 min. and then allowed to reach RT. A solution of DCC (206 mg, 1 mM) in DCM (10 cm^3) was added slowly over 1.25 hr. After stirring at RT for a further 20 hr., the reaction mixture was filtered to remove DCU and the solvent evaporated under vacuum. The residue was subjected to medium pressure column chromatography (5% EtOH in $CHCl_3$), giving a white crystalline solid, 6a, (290 mg, 0.79 mM, 79% yield, mpt: 108-109°C.

Z-Glyt-Gly-Gly-OMe, 6b. Z-Glyt-Gly-OPh, 3f, (171 mg, 0.5 mM), glycine methyl ester hydro-chloride (63 mg, 0.5 mM) and TEA (51 mg, 1 equiv) were heated to reflux in IPA (25 cm^3) for 8.5 hr. (No apparent reaction occurred at 20°C). After cooling, the solvent was evaporated in vacuo and the orange-brown residue subjected to medium pressure column chromatography (30% EtOAc/DCM) to remove impurities. This gave 6b as a white solid (83 mg, 0.24 mM, 47% yield), mpt: 130.5-132.5°C.

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